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08/14/03

PTO/SB/16 (05-03)

Approved for use through 4/30/2003. OMB 0651-0032

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Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

MBCATs as Modifiers of the Beta-Catenin Pathway and Methods of Use

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ENCLOSED APPLICATION PARTS (check all that apply)

Specification Number of Pages

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return receipt postcard

Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

Applicant claims small entity status. See 37 CFR 1.27.

A check or money order is enclosed to cover the filing fees

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The Director is hereby authorized to charge filing

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

No.

Yes, the name of the U.S. Government agency and the Government contract number are: _____.

[Page 1 of 2]

Date

8/14/2003

Respectfully submitted,
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47,937

(if appropriate)

Docket Number:

EX03-053P

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Docket Number	EX03-053P
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[Page 2 of 2]

Number 1 of 1

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MBCATs AS MODIFIERS OF THE BETA-CATENIN PATHWAY AND METHODS OF USE

BACKGROUND OF THE INVENTION

The *Drosophila Melanogaster* Armadillo/beta-catenin protein is implicated in multiple cellular functions. The protein functions in cell signaling via the Wingless (Wg)/Wnt signaling pathway. It also functions as a cell adhesion protein at the cell membrane in a complex with E-cadherin and alpha-catenin (Cox et al. (1996) *J. Cell Biol.* 134: 133-148; Godt and Tepass (1998) *Nature* 395: 387-391; White et al. (1998) *J Cell Biol.* 140:183-195). These two roles of beta-catenin can be separated from each other (Orsulic and Peifer (1996) *J. Cell Biol.* 134: 1283-1300; Sanson et al. (1996) *Nature* 383: 627-630).

In Wingless cell signaling, beta-catenin levels are tightly regulated by a complex containing APC, Axin, and GSK3 beta /SGG/ZW3 (Peifer et al. (1994) *Development* 120: 369-380).

The Wingless/beta-catenin signaling pathway is frequently mutated in human cancers, particularly those of the colon. Mutations in the tumor suppressor gene APC, as well as point mutations in beta-catenin itself lead to the stabilization of the beta-catenin protein and inappropriate activation of this pathway.

The ability to manipulate the genomes of model organisms such as *Drosophila* provides a powerful means to analyze biochemical processes that, due to significant evolutionary conservation, have direct relevance to more complex vertebrate organisms. Due to a high level of gene and pathway conservation, the strong similarity of cellular processes, and the functional conservation of genes between these model organisms and mammals, identification of the involvement of novel genes in particular pathways and their functions in such model organisms can directly contribute to the understanding of the correlative pathways and methods of modulating them in mammals (see, for example, Mechler BM et al., 1985 *EMBO J* 4:1551-1557; Gateff E. 1982 *Adv. Cancer Res.* 37: 33-74; Watson KL., et al., 1994 *J Cell Sci.* 108: 19-33; Miklos GL, and Rubin GM. 1996 *Cell* 86:521-529; Wassarman DA, et al., 1995 *Curr Opin Gen Dev* 5: 44-50; and Booth DR. 1999 *Cancer*

Metastasis Rev. 18: 261-284). For example, a genetic screen can be carried out in an invertebrate model organism having underexpression (e.g. knockout) or overexpression of a gene (referred to as a “genetic entry point”) that yields a visible phenotype. Additional genes are mutated in a random or targeted manner. When a gene mutation changes the original phenotype caused by the mutation in the genetic entry point, the gene is identified as a “modifier” involved in the same or overlapping pathway as the genetic entry point. When the genetic entry point is an ortholog of a human gene implicated in a disease pathway, such as beta-catenin, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

All references cited herein, including patents, patent applications, publications, and sequence information in referenced Genbank identifier numbers, are incorporated herein in their entireties.

SUMMARY OF THE INVENTION

We have discovered genes that modify the beta-catenin pathway in *Drosophila*, and identified their human orthologs, hereinafter referred to as modifier of beta catenin (MBCAT). The invention provides methods for utilizing these beta-catenin modifier genes and polypeptides to identify MBCAT-modulating agents that are candidate therapeutic agents that can be used in the treatment of disorders associated with defective or impaired beta-catenin function and/or MBCAT function. Preferred MBCAT-modulating agents specifically bind to MBCAT polypeptides and restore beta-catenin function. Other preferred MBCAT-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress MBCAT gene expression or product activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

MBCAT modulating agents may be evaluated by any convenient *in vitro* or *in vivo* assay for molecular interaction with an MBCAT polypeptide or nucleic acid. In one embodiment, candidate MBCAT modulating agents are tested with an assay system comprising a MBCAT polypeptide or nucleic acid. Agents that produce a change in the activity of the assay system relative to controls are identified as candidate beta-catenin modulating agents. The assay system may be cell-based or

cell-free. MBCAT-modulating agents include MBCAT related proteins (e.g. dominant negative mutants, and biotherapeutics); MBCAT -specific antibodies; MBCAT -specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind to or interact with MBCAT or compete with MBCAT binding partner (e.g. by binding to an MBCAT binding partner). In one specific embodiment, a small molecule modulator is identified using a binding assay. In specific embodiments, the screening assay system is selected from an apoptosis assay, a cell proliferation assay, an angiogenesis assay, and a hypoxic induction assay.

In another embodiment, candidate beta-catenin pathway modulating agents are further tested using a second assay system that detects changes in the beta-catenin pathway, such as angiogenic, apoptotic, or cell proliferation changes produced by the originally identified candidate agent or an agent derived from the original agent. The second assay system may use cultured cells or non-human animals. In specific embodiments, the secondary assay system uses non-human animals, including animals predetermined to have a disease or disorder implicating the beta-catenin pathway, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

The invention further provides methods for modulating the MBCAT function and/or the beta-catenin pathway in a mammalian cell by contacting the mammalian cell with an agent that specifically binds a MBCAT polypeptide or nucleic acid. The agent may be a small molecule modulator, a nucleic acid modulator, or an antibody and may be administered to a mammalian animal predetermined to have a pathology associated the beta-catenin pathway.

DETAILED DESCRIPTION OF THE INVENTION

In a screen to identify enhancers and suppressors of the Wg signaling pathway, we generated activated beta -catenin models in *Drosophila* based on human tumor data (Polakis (2000) Genes and Development 14: 1837-1851). We identified modifiers of the Wg pathway and identified their orthologs. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, MBCAT genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the treatment of pathologies associated with a

defective beta-catenin signaling pathway, such as cancer. Table 1 (Example II) lists the modifiers and their orthologs.

In vitro and in vivo methods of assessing MBCAT function are provided herein. Modulation of the MBCAT or their respective binding partners is useful for understanding the association of the beta-catenin pathway and its members in normal and disease conditions and for developing diagnostics and therapeutic modalities for beta-catenin related pathologies. MBCAT-modulating agents that act by inhibiting or enhancing MBCAT expression, directly or indirectly, for example, by affecting an MBCAT function such as enzymatic (e.g., catalytic) or binding activity, can be identified using methods provided herein. MBCAT modulating agents are useful in diagnosis, therapy and pharmaceutical development.

Nucleic acids and polypeptides of the invention

Sequences related to MBCAT nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) or RefSeq number), shown in Table 1 and in the appended sequence listing.

The term “MBCAT polypeptide” refers to a full-length MBCAT protein or a functionally active fragment or derivative thereof. A “functionally active” MBCAT fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type MBCAT protein, such as antigenic or immunogenic activity, enzymatic activity, ability to bind natural cellular substrates, etc. The functional activity of MBCAT proteins, derivatives and fragments can be assayed by various methods known to one skilled in the art (Current Protocols in Protein Science (1998) Coligan *et al.*, eds., John Wiley & Sons, Inc., Somerset, New Jersey) and as further discussed below. In one embodiment, a functionally active MBCAT polypeptide is a MBCAT derivative capable of rescuing defective endogenous MBCAT activity, such as in cell based or animal assays; the rescuing derivative may be from the same or a different species. For purposes herein, functionally active fragments also include those fragments that comprise one or more structural domains of an MBCAT, such as a kinase domain or a binding domain. Protein domains can be identified using the PFAM program (Bateman A., *et al.*, Nucleic Acids Res, 1999, 27:260-2). Methods

for obtaining MBCAT polypeptides are also further described below. In some embodiments, preferred fragments are functionally active, domain-containing fragments comprising at least 25 contiguous amino acids, preferably at least 50, more preferably 75, and most preferably at least 100 contiguous amino acids of an MBCAT. In further preferred embodiments, the fragment comprises the entire functionally active domain.

The term "MBCAT nucleic acid" refers to a DNA or RNA molecule that encodes a MBCAT polypeptide. Preferably, the MBCAT polypeptide or nucleic acid or fragment thereof is from a human, but can also be an ortholog, or derivative thereof with at least 70% sequence identity, preferably at least 80%, more preferably 85%, still more preferably 90%, and most preferably at least 95% sequence identity with human MBCAT. Methods of identifying orthologs are known in the art. Normally, orthologs in different species retain the same function, due to presence of one or more protein motifs and/or 3-dimensional structures. Orthologs are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential ortholog if the best hit sequence from the forward BLAST result retrieves the original query sequence in the reverse BLAST (Huynen MA and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen MA *et al.*, Genome Research (2000) 10:1204-1210). Programs for multiple sequence alignment, such as CLUSTAL (Thompson JD *et al*, 1994, Nucleic Acids Res 22:4673-4680) may be used to highlight conserved regions and/or residues of orthologous proteins and to generate phylogenetic trees. In a phylogenetic tree representing multiple homologous sequences from diverse species (e.g., retrieved through BLAST analysis), orthologous sequences from two species generally appear closest on the tree with respect to all other sequences from these two species. Structural threading or other analysis of protein folding (e.g., using software by ProCeryon, Biosciences, Salzburg, Austria) may also identify potential orthologs. In evolution, when a gene duplication event follows speciation, a single gene in one species, such as *Drosophila*, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a

specified portion of a subject sequence, is defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul *et al.*, *J. Mol. Biol.* (1997) 215:403-410) with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported. "Percent (%) amino acid sequence similarity" is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

A conservative amino acid substitution is one in which an amino acid is substituted for another amino acid having similar properties such that the folding or activity of the protein is not significantly affected. Aromatic amino acids that can be substituted for each other are phenylalanine, tryptophan, and tyrosine; interchangeable hydrophobic amino acids are leucine, isoleucine, methionine, and valine; interchangeable polar amino acids are glutamine and asparagine; interchangeable basic amino acids are arginine, lysine and histidine; interchangeable acidic amino acids are aspartic acid and glutamic acid; and interchangeable small amino acids are alanine, serine, threonine, cysteine and glycine.

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, *Advances in Applied Mathematics* 2:482-489; database: European Bioinformatics Institute; Smith and Waterman, 1981, *J. of Molec.Biol.*, 147:195-197; Nicholas *et al.*, 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.; W.R. Pearson, 1991, *Genomics* 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring

matrix developed by Dayhoff (Dayhoff: *Atlas of Protein Sequences and Structure*, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 *Nucl. Acids Res.* 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of an MBCAT. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (e.g., *Current Protocol in Molecular Biology*, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook *et al.*, *Molecular Cloning*, Cold Spring Harbor (1989)). In some embodiments, a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of an MBCAT under high stringency hybridization conditions that are: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 µg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100 µg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1h in a solution containing 0.1X SSC and 0.1% SDS (sodium dodecyl sulfate).

In other embodiments, moderately stringent hybridization conditions are used that are: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA; hybridization for 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100

μ g/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

Alternatively, low stringency conditions can be used that are: incubation for 8 hours to overnight at 37° C in a solution comprising 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 μ g/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in 1 x SSC at about 37° C for 1 hour.

Isolation, Production, Expression, and Mis-expression of MBCAT

Nucleic Acids and Polypeptides

MBCAT nucleic acids and polypeptides, are useful for identifying and testing agents that modulate MBCAT function and for other applications related to the involvement of MBCAT in the beta-catenin pathway. MBCAT nucleic acids and derivatives and orthologs thereof may be obtained using any available method. For instance, techniques for isolating cDNA or genomic DNA sequences of interest by screening DNA libraries or by using polymerase chain reaction (PCR) are well known in the art. In general, the particular use for the protein will dictate the particulars of expression, production, and purification methods. For instance, production of proteins for use in screening for modulating agents may require methods that preserve specific biological activities of these proteins, whereas production of proteins for antibody generation may require structural integrity of particular epitopes.

Expression of proteins to be purified for screening or antibody production may require the addition of specific tags (e.g., generation of fusion proteins).

Overexpression of an MBCAT protein for assays used to assess MBCAT function, such as involvement in cell cycle regulation or hypoxic response, may require expression in eukaryotic cell lines capable of these cellular activities. Techniques for the expression, production, and purification of proteins are well known in the art; any suitable means therefore may be used (e.g., Higgins SJ and Hames BD (eds.) *Protein Expression: A Practical Approach*, Oxford University Press Inc., New York 1999; Stanbury PF et al., *Principles of Fermentation Technology*, 2nd edition, Elsevier Science, New York, 1995; Doonan S (ed.) *Protein Purification Protocols*, Humana

Press, New Jersey, 1996; Coligan JE et al, Current Protocols in Protein Science (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant MBCAT is expressed in a cell line known to have defective beta-catenin function. The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

The nucleotide sequence encoding an MBCAT polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native MBCAT gene and/or its flanking regions or can be heterologous. A variety of host-vector expression systems may be utilized, such as mammalian cell systems infected with virus (e.g. vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g. baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, plasmid, or cosmid DNA. An isolated host cell strain that modulates the expression of, modifies, and/or specifically processes the gene product may be used.

To detect expression of the MBCAT gene product, the expression vector can comprise a promoter operably linked to an MBCAT gene nucleic acid, one or more origins of replication, and, one or more selectable markers (e.g. thymidine kinase activity, resistance to antibiotics, etc.). Alternatively, recombinant expression vectors can be identified by assaying for the expression of the MBCAT gene product based on the physical or functional properties of the MBCAT protein in *in vitro* assay systems (e.g. immunoassays).

The MBCAT protein, fragment, or derivative may be optionally expressed as a fusion, or chimeric protein product (i.e. it is joined via a peptide bond to a heterologous protein sequence of a different protein), for example to facilitate purification or detection. A chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other using standard methods and expressing the chimeric product. A chimeric product may also be made by protein synthetic techniques, e.g. by use of a peptide synthesizer (Hunkapiller *et al.*, *Nature* (1984) 310:105-111).

Once a recombinant cell that expresses the MBCAT gene sequence is identified, the gene product can be isolated and purified using standard methods (e.g. ion exchange, affinity, and gel exclusion chromatography; centrifugation; differential solubility; electrophoresis). Alternatively, native MBCAT proteins can be purified from natural sources, by standard methods (e.g. immunoaffinity purification). Once a protein is obtained, it may be quantified and its activity measured by appropriate methods, such as immunoassay, bioassay, or other measurements of physical properties, such as crystallography.

The methods of this invention may also use cells that have been engineered for altered expression (mis-expression) of MBCAT or other genes associated with the beta-catenin pathway. As used herein, mis-expression encompasses ectopic expression, over-expression, under-expression, and non-expression (e.g. by gene knock-out or blocking expression that would otherwise normally occur).

Genetically modified animals

Animal models that have been genetically modified to alter MBCAT expression may be used in *in vivo* assays to test for activity of a candidate beta-catenin modulating agent, or to further assess the role of MBCAT in a beta-catenin pathway process such as apoptosis or cell proliferation. Preferably, the altered MBCAT expression results in a detectable phenotype, such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal MBCAT expression. The genetically modified animal may additionally have altered beta-catenin expression (e.g. beta-catenin knockout). Preferred genetically modified animals are mammals such as primates, rodents (preferably mice or rats), among others. Preferred non-mammalian species include zebrafish, *C. elegans*, and *Drosophila*. Preferred genetically modified animals are transgenic animals having a heterologous nucleic acid sequence present as an extrachromosomal element in a portion of its cells, i.e. mosaic animals (see, for example, techniques described by Jakobovits, 1994, *Curr. Biol.* 4:761-763.) or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic acid is

introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal.

Methods of making transgenic animals are well-known in the art (for transgenic mice see Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985), U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al., and Hogan, B., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); for particle bombardment see U.S. Pat. No., 4,945,050, by Sandford *et al.*; for transgenic *Drosophila* see Rubin and Spradling, Science (1982) 218:348-53 and U.S. Pat. No. 4,670,388; for transgenic insects see Berghammer A.J. *et al.*, A Universal Marker for Transgenic Insects (1999) Nature 402:370-371; for transgenic Zebrafish see Lin S., Transgenic Zebrafish, Methods Mol Biol. (2000);136:375-3830); for microinjection procedures for fish, amphibian eggs and birds see Houdebine and Chourrout, Experientia (1991) 47:897-905; for transgenic rats see Hammer *et al.*, Cell (1990) 63:1099-1112; and for culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection see, e.g., Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E. J. Robertson, ed., IRL Press (1987)). Clones of the nonhuman transgenic animals can be produced according to available methods (see Wilmut, I. *et al.* (1997) Nature 385:810-813; and PCT International Publication Nos. WO 97/07668 and WO 97/07669).

In one embodiment, the transgenic animal is a “knock-out” animal having a heterozygous or homozygous alteration in the sequence of an endogenous MBCAT gene that results in a decrease of MBCAT function, preferably such that MBCAT expression is undetectable or insignificant. Knock-out animals are typically generated by homologous recombination with a vector comprising a transgene having at least a portion of the gene to be knocked out. Typically a deletion, addition or substitution has been introduced into the transgene to functionally disrupt it. The transgene can be a human gene (e.g., from a human genomic clone) but more preferably is an ortholog of the human gene derived from the transgenic host species. For example, a mouse MBCAT gene is used to construct a homologous

recombination vector suitable for altering an endogenous MBCAT gene in the mouse genome. Detailed methodologies for homologous recombination in mice are available (see Capecchi, *Science* (1989) 244:1288-1292; Joyner *et al.*, *Nature* (1989) 338:153-156). Procedures for the production of non-rodent transgenic mammals and other animals are also available (Houdebine and Chourrout, *supra*; Pursel *et al.*, *Science* (1989) 244:1281-1288; Simms *et al.*, *Bio/Technology* (1988) 6:179-183). In a preferred embodiment, knock-out animals, such as mice harboring a knockout of a specific gene, may be used to produce antibodies against the human counterpart of the gene that has been knocked out (Claesson MH *et al.*, (1994) *Scan J Immunol* 40:257-264; Declerck PJ *et al.*, (1995) *J Biol Chem*. 270:8397-400).

In another embodiment, the transgenic animal is a "knock-in" animal having an alteration in its genome that results in altered expression (e.g., increased (including ectopic) or decreased expression) of the MBCAT gene, e.g., by introduction of additional copies of MBCAT, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the MBCAT gene. Such regulatory sequences include inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

Transgenic nonhuman animals can also be produced that contain selected systems allowing for regulated expression of the transgene. One example of such a system that may be produced is the cre/loxP recombinase system of bacteriophage P1 (Lakso *et al.*, *PNAS* (1992) 89:6232-6236; U.S. Pat. No. 4,959,317). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.*, (1991) *Science* 251:1351-1355; U.S. Pat. No. 5,654,182). In a preferred embodiment, both Cre-LoxP and Flp-Frt are used in the same system to regulate expression of the transgene, and for sequential deletion of vector sequences in the same cell (Sun X *et al* (2000) *Nat Genet* 25:83-6).

The genetically modified animals can be used in genetic studies to further elucidate the beta-catenin pathway, as animal models of disease and disorders implicating defective beta-catenin function, and for *in vivo* testing of candidate therapeutic agents, such as those identified in screens described below. The candidate therapeutic agents are administered to a genetically modified animal having altered MBCAT function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered MBCAT expression that receive candidate therapeutic agent.

In addition to the above-described genetically modified animals having altered MBCAT function, animal models having defective beta-catenin function (and otherwise normal MBCAT function), can be used in the methods of the present invention. For example, a beta-catenin knockout mouse can be used to assess, *in vivo*, the activity of a candidate beta-catenin modulating agent identified in one of the *in vitro* assays described below. Preferably, the candidate beta-catenin modulating agent when administered to a model system with cells defective in beta-catenin function, produces a detectable phenotypic change in the model system indicating that the beta-catenin function is restored, i.e., the cells exhibit normal cell cycle progression.

Modulating Agents

The invention provides methods to identify agents that interact with and/or modulate the function of MBCAT and/or the beta-catenin pathway. Modulating agents identified by the methods are also part of the invention. Such agents are useful in a variety of diagnostic and therapeutic applications associated with the beta-catenin pathway, as well as in further analysis of the MBCAT protein and its contribution to the beta-catenin pathway. Accordingly, the invention also provides methods for modulating the beta-catenin pathway comprising the step of specifically modulating MBCAT activity by administering a MBCAT-interacting or -modulating agent.

As used herein, an "MBCAT-modulating agent" is any agent that modulates MBCAT function, for example, an agent that interacts with MBCAT to inhibit or enhance MBCAT activity or otherwise affect normal MBCAT function. MBCAT function can be affected at any level, including transcription, protein expression, protein localization, and

cellular or extra-cellular activity. In a preferred embodiment, the MBCAT - modulating agent specifically modulates the function of the MBCAT. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the MBCAT polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the MBCAT. These phrases also encompass modulating agents that alter the interaction of the MBCAT with a binding partner, substrate, or cofactor (e.g. by binding to a binding partner of an MBCAT, or to a protein/binding partner complex, and altering MBCAT function). In a further preferred embodiment, the MBCAT- modulating agent is a modulator of the beta-catenin pathway (e.g. it restores and/or upregulates beta-catenin function) and thus is also a beta-catenin-modulating agent.

Preferred MBCAT-modulating agents include small molecule compounds; MBCAT-interacting proteins, including antibodies and other biotherapeutics; and nucleic acid modulators such as antisense and RNA inhibitors. The modulating agents may be formulated in pharmaceutical compositions, for example, as compositions that may comprise other active ingredients, as in combination therapy, and/or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, 19th edition.

Small molecule modulators

Small molecules are often preferred to modulate function of proteins with enzymatic function, and/or containing protein interaction domains. Chemical agents, referred to in the art as "small molecule" compounds are typically organic, non-peptide molecules, having a molecular weight less than 10,000, preferably less than 5,000, more preferably less than 1,000, and most preferably less than 500 daltons. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the MBCAT protein or may be identified by screening compound libraries. Alternative appropriate modulators of this class are natural products, particularly secondary metabolites from organisms such as plants or fungi, which can

also be identified by screening compound libraries for MBCAT-modulating activity. Methods for generating and obtaining compounds are well known in the art (Schreiber SL, *Science* (2000) 151: 1964-1969; Radmann J and Gunther J, *Science* (2000) 151:1947-1948).

Small molecule modulators identified from screening assays, as described below, can be used as lead compounds from which candidate clinical compounds may be designed, optimized, and synthesized. Such clinical compounds may have utility in treating pathologies associated with the beta-catenin pathway. The activity of candidate small molecule modulating agents may be improved several-fold through iterative secondary functional validation, as further described below, structure determination, and candidate modulator modification and testing. Additionally, candidate clinical compounds are generated with specific regard to clinical and pharmacological properties. For example, the reagents may be derivatized and re-screened using *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

Protein Modulators

Specific MBCAT-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the beta-catenin pathway and related disorders, as well as in validation assays for other MBCAT-modulating agents. In a preferred embodiment, MBCAT-interacting proteins affect normal MBCAT function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, MBCAT-interacting proteins are useful in detecting and providing information about the function of MBCAT proteins, as is relevant to beta-catenin related disorders, such as cancer (e.g., for diagnostic means).

An MBCAT-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an MBCAT, such as a member of the MBCAT pathway that modulates MBCAT expression, localization, and/or activity. MBCAT-modulators include dominant negative forms of MBCAT-interacting proteins and of MBCAT proteins themselves. Yeast two-hybrid and variant screens offer preferred methods for identifying endogenous MBCAT-interacting proteins (Finley, R. L. et al. (1996) in *DNA Cloning-Expression Systems: A Practical Approach*, eds. Glover D. &

Hames B. D (Oxford University Press, Oxford, England), pp. 169-203; Fashema SF et al., Gene (2000) 250:1-14; Drees BL Curr Opin Chem Biol (1999) 3:64-70; Vidal M and Legrain P Nucleic Acids Res (1999) 27:919-29; and U.S. Pat. No. 5,928,868). Mass spectrometry is an alternative preferred method for the elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, Nature (2000) 405:837-846; Yates JR 3rd, Trends Genet (2000) 16:5-8).

An MBCAT-interacting protein may be an exogenous protein, such as an MBCAT-specific antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory; Harlow and Lane (1999) *Using antibodies: a laboratory manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). MBCAT antibodies are further discussed below.

In preferred embodiments, an MBCAT-interacting protein specifically binds an MBCAT protein. In alternative preferred embodiments, an MBCAT-modulating agent binds an MBCAT substrate, binding partner, or cofactor.

Antibodies

In another embodiment, the protein modulator is an MBCAT specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify MBCAT modulators. The antibodies can also be used in dissecting the portions of the MBCAT pathway responsible for various cellular responses and in the general processing and maturation of the MBCAT.

Antibodies that specifically bind MBCAT polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of MBCAT polypeptide, and more preferably, to human MBCAT. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Epitopes of MBCAT which are particularly antigenic can be selected, for example, by routine screening of MBCAT polypeptides for antigenicity or by applying a theoretical method for selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. Natl. Acad. Sci. U.S.A. 78:3824-28; Hopp and Wood, (1983) Mol. Immunol. 20:483-89;

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Sutcliffe et al., (1983) *Science* 219:660-66) to the amino acid sequence of an MBCAT. Monoclonal antibodies with affinities of 10^8 M⁻¹ preferably 10^9 M⁻¹ to 10^{10} M⁻¹, or stronger can be made by standard procedures as described (Harlow and Lane, *supra*; Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577). Antibodies may be generated against crude cell extracts of MBCAT or substantially purified fragments thereof. If MBCAT fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of an MBCAT protein. In a particular embodiment, MBCAT-specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate immune system such as a laboratory rabbit or mouse is immunized according to conventional protocols.

The presence of MBCAT-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized corresponding MBCAT polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.

Chimeric antibodies specific to MBCAT polypeptides can be made that contain different portions from different animal species. For instance, a human immunoglobulin constant region may be linked to a variable region of a murine mAb, such that the antibody derives its biological activity from the human antibody, and its binding specificity from the murine fragment. Chimeric antibodies are produced by splicing together genes that encode the appropriate regions from each species (Morrison et al., *Proc. Natl. Acad. Sci.* (1984) 81:6851-6855; Neuberger et al., *Nature* (1984) 312:604-608; Takeda et al., *Nature* (1985) 31:452-454). Humanized antibodies, which are a form of chimeric antibodies, can be generated by grafting complementary-determining regions (CDRs) (Carlos, T. M., J. M. Harlan. 1994. *Blood* 84:2068-2101) of mouse antibodies into a background of human framework regions and constant regions by recombinant DNA technology (Riechmann LM, et al., 1988 *Nature* 323: 323-327). Humanized antibodies contain ~10% murine sequences and ~90% human sequences, and thus further

reduce or eliminate immunogenicity, while retaining the antibody specificities (Co MS, and Queen C. 1991 *Nature* 351: 501-501; Morrison SL. 1992 *Ann. Rev. Immun.* 10:239-265). Humanized antibodies and methods of their production are well-known in the art (U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,762, and 6,180,370).

MBCAT-specific single chain antibodies which are recombinant, single chain polypeptides formed by linking the heavy and light chain fragments of the Fv regions via an amino acid bridge, can be produced by methods known in the art (U.S. Pat. No. 4,946,778; Bird, *Science* (1988) 242:423-426; Huston et al., *Proc. Natl. Acad. Sci. USA* (1988) 85:5879-5883; and Ward et al., *Nature* (1989) 334:544-546).

Other suitable techniques for antibody production involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors (Huse et al., *Science* (1989) 246:1275-1281). As used herein, T-cell antigen receptors are included within the scope of antibody modulators (Harlow and Lane, 1988, *supra*).

The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently or non-covalently, a substance that provides for a detectable signal, or that is toxic to cells that express the targeted protein (Menard S, et al., *Int J. Biol Markers* (1989) 4:131-134). A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, fluorescent emitting lanthanide metals, chemiluminescent moieties, bioluminescent moieties, magnetic particles, and the like (U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241). Also, recombinant immunoglobulins may be produced (U.S. Pat. No. 4,816,567). Antibodies to cytoplasmic polypeptides may be delivered and reach their targets by conjugation with membrane-penetrating toxin proteins (U.S. Pat. No. 6,086,900).

When used therapeutically in a patient, the antibodies of the subject invention are typically administered parenterally, when possible at the target site, or intravenously. The therapeutically effective dose and dosage regimen is determined by clinical studies. Typically, the amount of antibody administered is in the range of about 0.1 mg/kg -to

about 10 mg/kg of patient weight. For parenteral administration, the antibodies are formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion) in association with a pharmaceutically acceptable vehicle. Such vehicles are inherently nontoxic and non-therapeutic. Examples are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils, ethyl oleate, or liposome carriers may also be used. The vehicle may contain minor amounts of additives, such as buffers and preservatives, which enhance isotonicity and chemical stability or otherwise enhance therapeutic potential. The antibodies' concentrations in such vehicles are typically in the range of about 1 mg/ml to about 10 mg/ml.

Immunotherapeutic methods are further described in the literature (US Pat. No. 5,859,206; WO0073469).

Specific biotherapeutics

In a preferred embodiment, an MBCAT-interacting protein may have biotherapeutic applications. Biotherapeutic agents formulated in pharmaceutically acceptable carriers and dosages may be used to activate or inhibit signal transduction pathways. This modulation may be accomplished by binding a ligand, thus inhibiting the activity of the pathway; or by binding a receptor, either to inhibit activation of, or to activate, the receptor. Alternatively, the biotherapeutic may itself be a ligand capable of activating or inhibiting a receptor. Biotherapeutic agents and methods of producing them are described in detail in U.S. Pat. No. 6,146,628.

When the MBCAT is a ligand, it may be used as a biotherapeutic agent to activate or inhibit its natural receptor. Alternatively, antibodies against MBCAT, as described in the previous section, may be used as biotherapeutic agents.

When the MBCAT is a receptor, its ligand(s), antibodies to the ligand(s) or the MBCAT itself may be used as biotherapeutics to modulate the activity of MBCAT in the beta-catenin pathway.

Nucleic Acid Modulators

Other preferred MBCAT-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit MBCAT

activity. Preferred nucleic acid modulators interfere with the function of the MBCAT nucleic acid such as DNA replication, transcription, translocation of the MBCAT RNA to the site of protein translation, translation of protein from the MBCAT RNA, splicing of the MBCAT RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the MBCAT RNA.

In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to an MBCAT mRNA to bind to and prevent translation, preferably by binding to the 5' untranslated region. MBCAT-specific antisense oligonucleotides, preferably range from at least 6 to about 200 nucleotides. In some embodiments the oligonucleotide is preferably at least 10, 15, or 20 nucleotides in length. In other embodiments, the oligonucleotide is preferably less than 50, 40, or 30 nucleotides in length. The oligonucleotide can be DNA or RNA or a chimeric mixture or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, agents that facilitate transport across the cell membrane, hybridization-triggered cleavage agents, and intercalating agents.

In another embodiment, the antisense oligomer is a phosphothioate morpholino oligomer (PMO). PMOs are assembled from four different morpholino subunits, each of which contain one of four genetic bases (A, C, G, or T) linked to a six-membered morpholine ring. Polymers of these subunits are joined by non-ionic phosphodiamidate intersubunit linkages. Details of how to make and use PMOs and other antisense oligomers are well known in the art (e.g. see WO99/18193; Probst JC, Antisense Oligodeoxynucleotide and Ribozyme Design, Methods. (2000) 22(3):271-281; Summerton J, and Weller D. 1997 Antisense Nucleic Acid Drug Dev. :7:187-95; US Pat. No. 5,235,033; and US Pat No. 5,378,841).

Alternative preferred MBCAT nucleic acid modulators are double-stranded RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in *C. elegans*, *Drosophila*, plants, and humans are

known in the art (Fire A, et al., 1998 *Nature* 391:806-811; Fire, A. *Trends Genet.* 15, 358-363 (1999); Sharp, P. A. *RNA interference* 2001. *Genes Dev.* 15, 485-490 (2001); Hammond, S. M., et al., *Nature Rev. Genet.* 2, 110-1119 (2001); Tuschl, T. *Chem. Biochem.* 2, 239-245 (2001); Hamilton, A. et al., *Science* 286, 950-952 (1999); Hammond, S. M., et al., *Nature* 404, 293-296 (2000); Zamore, P. D., et al., *Cell* 101, 25-33 (2000); Bernstein, E., et al., *Nature* 409, 363-366 (2001); Elbashir, S. M., et al., *Genes Dev.* 15, 188-200 (2001); WO0129058; WO9932619; Elbashir SM, et al., 2001 *Nature* 411:494-498).

Nucleic acid modulators are commonly used as research reagents, diagnostics, and therapeutics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used to elucidate the function of particular genes (see, for example, U.S. Pat. No. 6,165,790). Nucleic acid modulators are also used, for example, to distinguish between functions of various members of a biological pathway. For example, antisense oligomers have been employed as therapeutic moieties in the treatment of disease states in animals and man and have been demonstrated in numerous clinical trials to be safe and effective (Milligan JF, *et al.*, *Current Concepts in Antisense Drug Design*, *J Med Chem.* (1993) 36:1923-1937; Tonkinson JL *et al.*, *Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents*, *Cancer Invest.* (1996) 14:54-65). Accordingly, in one aspect of the invention, an MBCAT-specific nucleic acid modulator is used in an assay to further elucidate the role of the MBCAT in the beta-catenin pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, an MBCAT-specific antisense oligomer is used as a therapeutic agent for treatment of beta-catenin-related disease states.

Assay Systems

The invention provides assay systems and screening methods for identifying specific modulators of MBCAT activity. As used herein, an "assay system" encompasses all the components required for performing and analyzing results of an assay that detects and/or measures a particular event. In general, primary assays are used to identify or confirm a modulator's specific biochemical or molecular effect with respect to the MBCAT nucleic acid or protein. In general, secondary assays further assess the activity of a MBCAT

modulating agent identified by a primary assay and may confirm that the modulating agent affects MBCAT in a manner relevant to the beta-catenin pathway. In some cases, MBCAT modulators will be directly tested in a secondary assay.

In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising an MBCAT polypeptide or nucleic acid with a candidate agent under conditions whereby, but for the presence of the agent, the system provides a reference activity (e.g. kinase activity), which is based on the particular molecular event the screening method detects. A statistically significant difference between the agent-biased activity and the reference activity indicates that the candidate agent modulates MBCAT activity, and hence the beta-catenin pathway. The MBCAT polypeptide or nucleic acid used in the assay may comprise any of the nucleic acids or polypeptides described above.

Primary Assays

The type of modulator tested generally determines the type of primary assay.

Primary assays for small molecule modulators

For small molecule modulators, screening assays are used to identify candidate modulators. Screening assays may be cell-based or may use a cell-free system that recreates or retains the relevant biochemical reaction of the target protein (reviewed in Sittampalam GS *et al.*, *Curr Opin Chem Biol* (1997) 1:384-91 and accompanying references). As used herein the term "cell-based" refers to assays using live cells, dead cells, or a particular cellular fraction, such as a membrane, endoplasmic reticulum, or mitochondrial fraction. The term "cell free" encompasses assays using substantially purified protein (either endogenous or recombinantly produced), partially purified or crude cellular extracts. Screening assays may detect a variety of molecular events, including protein-DNA interactions, protein-protein interactions (e.g., receptor-ligand binding), transcriptional activity (e.g., using a reporter gene), enzymatic activity (e.g., via a property of the substrate), activity of second messengers, immunogenicity and changes in cellular morphology or other cellular characteristics. Appropriate screening assays may use a wide range of detection methods including fluorescent, radioactive,

colorimetric, spectrophotometric, and amperometric methods, to provide a read-out for the particular molecular event detected.

Cell-based screening assays usually require systems for recombinant expression of MBCAT and any auxiliary proteins demanded by the particular assay. Appropriate methods for generating recombinant proteins produce sufficient quantities of proteins that retain their relevant biological activities and are of sufficient purity to optimize activity and assure assay reproducibility. Yeast two-hybrid and variant screens, and mass spectrometry provide preferred methods for determining protein-protein interactions and elucidation of protein complexes. In certain applications, when MBCAT-interacting proteins are used in screens to identify small molecule modulators, the binding specificity of the interacting protein to the MBCAT protein may be assayed by various known methods such as substrate processing (e.g. ability of the candidate MBCAT-specific binding agents to function as negative effectors in MBCAT-expressing cells), binding equilibrium constants (usually at least about 10^7 M^{-1} , preferably at least about 10^8 M^{-1} , more preferably at least about 10^9 M^{-1}), and immunogenicity (e.g. ability to elicit MBCAT specific antibody in a heterologous host such as a mouse, rat, goat or rabbit). For enzymes and receptors, binding may be assayed by, respectively, substrate and ligand processing.

The screening assay may measure a candidate agent's ability to specifically bind to or modulate activity of a MBCAT polypeptide, a fusion protein thereof, or to cells or membranes bearing the polypeptide or fusion protein. The MBCAT polypeptide can be full length or a fragment thereof that retains functional MBCAT activity. The MBCAT polypeptide may be fused to another polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The MBCAT polypeptide is preferably human MBCAT, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-based modulation of MBCAT interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has MBCAT -specific binding activity, and can be used to assess normal MBCAT gene function.

Suitable assay formats that may be adapted to screen for MBCAT modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput

and thus provide automated, cost-effective means of screening compound libraries for lead compounds (Fernandes PB, *Curr Opin Chem Biol* (1998) 2:597-603; Sundberg SA, *Curr Opin Biotechnol* 2000, 11:47-53). In one preferred embodiment, screening assays uses fluorescence technologies, including fluorescence polarization, time-resolved fluorescence, and fluorescence resonance energy transfer. These systems offer means to monitor protein-protein or DNA-protein interactions in which the intensity of the signal emitted from dye-labeled molecules depends upon their interactions with partner molecules (e.g., Selvin PR, *Nat Struct Biol* (2000) 7:730-4; Fernandes PB, *supra*; Hertzberg RP and Pope AJ, *Curr Opin Chem Biol* (2000) 4:445-451).

A variety of suitable assay systems may be used to identify candidate MBCAT and beta-catenin pathway modulators (e.g. U.S. Pat. No. 6,165,992 (kinase assays); U.S. Pat. Nos. 5,550,019 and 6,133,437 (apoptosis assays); and U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434 (angiogenesis assays), among others). Specific preferred assays are described in more detail below.

Protein kinases, key signal transduction proteins that may be either membrane-associated or intracellular, catalyze the transfer of gamma phosphate from adenosine triphosphate (ATP) to a serine, threonine or tyrosine residue in a protein substrate. Radioassays, which monitor the transfer from [γ -³²P or ³³P]ATP, are frequently used to assay kinase activity. For instance, a scintillation assay for p56 (lck) kinase activity monitors the transfer of the gamma phosphate from [γ -³³P] ATP to a biotinylated peptide substrate. The substrate is captured on a streptavidin coated bead that transmits the signal (Beveridge M *et al.*, *J Biomol Screen* (2000) 5:205-212). This assay uses the scintillation proximity assay (SPA), in which only radio-ligand bound to receptors tethered to the surface of an SPA bead are detected by the scintillant immobilized within it, allowing binding to be measured without separation of bound from free ligand. Other assays for protein kinase activity may use antibodies that specifically recognize phosphorylated substrates. For instance, the kinase receptor activation (KIRA) assay measures receptor tyrosine kinase activity by ligand stimulating the intact receptor in cultured cells, then capturing solubilized receptor with specific antibodies and quantifying phosphorylation via phosphotyrosine ELISA (Sadick MD, *Dev Biol Stand* (1999) 97:121-133). Another example of antibody based assays for protein kinase

activity is TRF (time-resolved fluorometry). This method utilizes europium chelate-labeled anti-phosphotyrosine antibodies to detect phosphate transfer to a polymeric substrate coated onto microtiter plate wells. The amount of phosphorylation is then detected using time-resolved, dissociation-enhanced fluorescence (Braunwalder AF, et al., *Anal Biochem* 1996 Jul 1;238(2):159-64).

Transporter proteins carry a range of substrates, including nutrients, ions, amino acids, and drugs, across cell membranes. Assays for modulators of transporters may use labeled substrates. For instance, exemplary high throughput screens to identify compounds that interact with different peptide and anion transporters both use fluorescently labeled substrates; the assay for peptide transport additionally uses multiscreen filtration plates (Blevitt JM et al., *J Biomol Screen* 1999, 4:87-91; Cihlar T and Ho ES, *Anal Biochem* 2000, 283:49-55).

Ion channels mediate essential physiological functions, including fluid secretion, electrolyte balance, bioenergetics, and membrane excitability. Assays for channel activity can incorporate ion-sensitive dyes or proteins or voltage-sensitive dyes or proteins, as reviewed in Gonzalez JE *et al.* (*Drug Discovery Today* (1999) 4:431-439). Alternative methods measure the displacement of known ligands, which may be radio-labeled or fluorescently labeled (e.g., Schmid EL *et al.*, *Anal Chem* (1998) 70:1331-1338).

Transcription factors control gene transcription. Electrophoretic mobility shift assay (EMSA) or gel shift assay is one of the most powerful methods for studying protein-DNA interactions. High throughput gel shift assays for transcription factors may involve fluorescence (Cyano dye Cy5) labeled oligodeoxynucleotide duplexes as specific probes and an automatic DNA sequencer for analysis (Ruscher K, et al., (2000) *J Biotechnol* 78:163-70). Alternatively high throughput methods involve colorimetric assays (Renard P, et al. (2001) *Nucleic Acids Res* 29(4):E21), or homogeneous fluorescence assays for the detection and quantification of sequence-specific DNA-binding proteins (Heyduk T, and Heyduk E (2001) *Nat Biotechnol* 20:171-6.)

High throughput assays based on photometric analysis of the activity of decarboxylase enzymes have been described (Breuer M et al (2002) *Anal Bioanal Chem* 374:1069-73).

High-throughput photometric assays for peroxidases have been described (Smith AD et al (2001) *Int J Vitam Nutr Res* 71:87-92; Smith AD and Levander OA (2002) *Methods Enzymol* 347:113-21).

High throughput adenylyl cyclase assays are known in the art, and are also commercially available (NEN® Adenylyl Cyclase Activation FlashPlate® Assay, available from Perkin Elmer Life Sciences, Boston, MA). Examples include homogeneous cellular assays that allow direct measurement of receptor-mediated adenylyl cyclase activation/inhibition.

Apoptosis assays. Assays for apoptosis may be performed by terminal deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay. The TUNEL assay is used to measure nuclear DNA fragmentation characteristic of apoptosis (Lazebnik *et al.*, 1994, *Nature* 371, 346), by following the incorporation of fluorescein-dUTP (Yonehara *et al.*, 1989, *J. Exp. Med.* 169, 1747). Apoptosis may further be assayed by acridine orange staining of tissue culture cells (Lucas, R., *et al.*, 1998, *Blood* 15:4730-41). Other cell-based apoptosis assays include the caspase-3/7 assay and the cell death nucleosome ELISA assay. The caspase 3/7 assay is based on the activation of the caspase cleavage activity as part of a cascade of events that occur during programmed cell death in many apoptotic pathways. In the caspase 3/7 assay (commercially available Apo-ONE™ Homogeneous Caspase-3/7 assay from Promega, cat# 67790), lysis buffer and caspase substrate are mixed and added to cells. The caspase substrate becomes fluorescent when cleaved by active caspase 3/7. The nucleosome ELISA assay is a general cell death assay known to those skilled in the art, and available commercially (Roche, Cat# 1774425). This assay is a quantitative sandwich-enzyme-immunoassay which uses monoclonal antibodies directed against DNA and histones respectively, thus specifically determining amount of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Mono and oligonucleosomes are enriched in the cytoplasm during apoptosis due to the fact that DNA fragmentation occurs several hours before the plasma membrane breaks down, allowing for accumulation in the cytoplasm. Nucleosomes are not present in the cytoplasmic fraction of cells that are not undergoing apoptosis. An apoptosis assay system may comprise a cell that expresses an MBCAT,

and that optionally has defective beta-catenin function (e.g. beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the apoptosis assay system and changes in induction of apoptosis relative to controls where no test agent is added, identify candidate beta-catenin modulating agents. In some embodiments of the invention, an apoptosis assay may be used as a secondary assay to test a candidate beta-catenin modulating agents that is initially identified using a cell-free assay system. An apoptosis assay may also be used to test whether MBCAT function plays a direct role in apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express MBCAT relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the MBCAT plays a direct role in the apoptotic response. Apoptosis assays are described further in US Pat. No. 6,133,437.

Cell proliferation and cell cycle assays. Cell proliferation may be assayed via bromodeoxyuridine (BRDU) incorporation. This assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly-synthesized DNA. Newly-synthesized DNA may then be detected using an anti-BRDU antibody (Hoshino *et al.*, 1986, Int. J. Cancer 38, 369; Campana *et al.*, 1988, J. Immunol. Meth. 107, 79), or by other means.

Cell proliferation is also assayed via phospho-histone H3 staining, which identifies a cell population undergoing mitosis by phosphorylation of histone H3. Phosphorylation of histone H3 at serine 10 is detected using an antibody specific to the phosphorylated form of the serine 10 residue of histone H3. (Chadlee,D.N. 1995, J. Biol. Chem 270:20098-105). Cell Proliferation may also be examined using [³H]-thymidine incorporation (Chen, J., 1996, Oncogene 13:1395-403; Jeoung, J., 1995, J. Biol. Chem. 270:18367-73). This assay allows for quantitative characterization of S-phase DNA syntheses. In this assay, cells synthesizing DNA will incorporate [³H]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of radioisotope in a scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation Counter). Another proliferation assay uses the dye Alamar Blue (available from Biosource International), which fluoresces when reduced in living cells and provides an

indirect measurement of cell number (Voytik-Harbin SL et al., 1998, *In Vitro Cell Dev Biol Anim* 34:239-46). Yet another proliferation assay, the MTS assay, is based on in vitro cytotoxicity assessment of industrial chemicals, and uses the soluble tetrazolium salt, MTS. MTS assays are commercially available, for example, the Promega CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Cat.# G5421).

Cell proliferation may also be assayed by colony formation in soft agar (Sambrook et al., *Molecular Cloning*, Cold Spring Harbor (1989)). For example, cells transformed with MBCAT are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

Cell proliferation may also be assayed by measuring ATP levels as indicator of metabolically active cells. Such assays are commercially available, for example Cell Titer-Glo™, which is a luminescent homogeneous assay available from Promega.

Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray JW et al. (1986) *Int J Radiat Biol Relat Stud Phys Chem Med* 49:237-55). Cells transfected with an MBCAT may be stained with propidium iodide and evaluated in a flow cytometer (available from Becton Dickinson), which indicates accumulation of cells in different stages of the cell cycle.

Accordingly, a cell proliferation or cell cycle assay system may comprise a cell that expresses an MBCAT, and that optionally has defective beta-catenin function (e.g. beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the assay system and changes in cell proliferation or cell cycle relative to controls where no test agent is added, identify candidate beta-catenin modulating agents. In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to test a candidate beta-catenin modulating agents that is initially identified using another assay system such as a cell-free assay system. A cell proliferation assay may also be used to test whether MBCAT function plays a direct role in cell proliferation or cell cycle. For example, a cell proliferation or cell cycle assay may be performed on cells that over- or under-express MBCAT relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the MBCAT plays a direct role in cell proliferation or cell cycle.

Angiogenesis. Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells through membranes in presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on Matrigel® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a cell that expresses an MBCAT, and that optionally has defective beta-catenin function (e.g. beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate beta-catenin modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate beta-catenin modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether MBCAT function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express MBCAT relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the MBCAT plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others, describe various angiogenesis assays.

Hypoxic induction. The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glyolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with MBCAT in hypoxic conditions (such as with 0.1% O₂, 5% CO₂, and balance N₂, generated in a Napco 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses an MBCAT, and that optionally has defective beta-catenin function (e.g. beta-catenin is

over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate beta-catenin modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate beta-catenin modulating agents that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether MBCAT function plays a direct role in the hypoxic response. For example, a hypoxic induction assay may be performed on cells that over- or under-express MBCAT relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the MBCAT plays a direct role in hypoxic induction.

Cell adhesion. Cell adhesion assays measure adhesion of cells to purified adhesion proteins, or adhesion of cells to each other, in presence or absence of candidate modulating agents. Cell-protein adhesion assays measure the ability of agents to modulate the adhesion of cells to purified proteins. For example, recombinant proteins are produced, diluted to 2.5g/mL in PBS, and used to coat the wells of a microtiter plate. The wells used for negative control are not coated. Coated wells are then washed, blocked with 1% BSA, and washed again. Compounds are diluted to 2× final test concentration and added to the blocked, coated wells. Cells are then added to the wells, and the unbound cells are washed off. Retained cells are labeled directly on the plate by adding a membrane-permeable fluorescent dye, such as calcein-AM, and the signal is quantified in a fluorescent microplate reader.

Cell-cell adhesion assays measure the ability of agents to modulate binding of cell adhesion proteins with their native ligands. These assays use cells that naturally or recombinantly express the adhesion protein of choice. In an exemplary assay, cells expressing the cell adhesion protein are plated in wells of a multiwell plate. Cells expressing the ligand are labeled with a membrane-permeable fluorescent dye, such as BCECF, and allowed to adhere to the monolayers in the presence of candidate agents. Unbound cells are washed off, and bound cells are detected using a fluorescence plate reader.

High-throughput cell adhesion assays have also been described. In one such assay, small molecule ligands and peptides are bound to the surface of microscope slides using a microarray spotter, intact cells are then contacted with the slides, and unbound cells are washed off. In this assay, not only the binding specificity of the peptides and modulators against cell lines are determined, but also the functional cell signaling of attached cells using immunofluorescence techniques *in situ* on the microchip is measured (Falsey JR et al., *Bioconjug Chem.* 2001 May-Jun;12(3):346-53).

Tubulogenesis. Tubulogenesis assays monitor the ability of cultured cells, generally endothelial cells, to form tubular structures on a matrix substrate, which generally simulates the environment of the extracellular matrix. Exemplary substrates include Matrigel™ (Becton Dickinson), an extract of basement membrane proteins containing laminin, collagen IV, and heparin sulfate proteoglycan, which is liquid at 4° C and forms a solid gel at 37° C. Other suitable matrices comprise extracellular components such as collagen, fibronectin, and/or fibrin. Cells are stimulated with a pro-angiogenic stimulant, and their ability to form tubules is detected by imaging. Tubules can generally be detected after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Tube formation assays are well known in the art (e.g., Jones MK et al., 1999, *Nature Medicine* 5:1418-1423). These assays have traditionally involved stimulation with serum or with the growth factors FGF or VEGF. Serum represents an undefined source of growth factors. In a preferred embodiment, the assay is performed with cells cultured in serum free medium, in order to control which process or pathway a candidate agent modulates. Moreover, we have found that different target genes respond differently to stimulation with different pro-angiogenic agents, including inflammatory angiogenic factors such as TNF-alpha. Thus, in a further preferred embodiment, a tubulogenesis assay system comprises testing an MBCAT's response to a variety of factors, such as FGF, VEGF, phorbol myristate acetate (PMA), TNF-alpha, ephrin, etc.

Cell Migration. An invasion/migration assay (also called a migration assay) tests the ability of cells to overcome a physical barrier and to migrate towards pro-angiogenic

signals. Migration assays are known in the art (e.g., Paik JH et al., 2001, J Biol Chem 276:11830-11837). In a typical experimental set-up, cultured endothelial cells are seeded onto a matrix-coated porous lamina, with pore sizes generally smaller than typical cell size. The matrix generally simulates the environment of the extracellular matrix, as described above. The lamina is typically a membrane, such as the transwell polycarbonate membrane (Corning Costar Corporation, Cambridge, MA), and is generally part of an upper chamber that is in fluid contact with a lower chamber containing pro-angiogenic stimuli. Migration is generally assayed after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Migration is assessed as the number of cells that crossed the lamina, and may be detected by staining cells with hemotoxylin solution (VWR Scientific, South San Francisco, CA), or by any other method for determining cell number. In another exemplary set up, cells are fluorescently labeled and migration is detected using fluorescent readings, for instance using the Falcon HTS FluoroBlok (Becton Dickinson). While some migration is observed in the absence of stimulus, migration is greatly increased in response to pro-angiogenic factors. As described above, a preferred assay system for migration/invasion assays comprises testing an MBCAT's response to a variety of pro-angiogenic factors, including tumor angiogenic and inflammatory angiogenic agents, and culturing the cells in serum free medium.

Sprouting assay. A sprouting assay is a three-dimensional *in vitro* angiogenesis assay that uses a cell-number defined spheroid aggregation of endothelial cells ("spheroid"), embedded in a collagen gel-based matrix. The spheroid can serve as a starting point for the sprouting of capillary-like structures by invasion into the extracellular matrix (termed "cell sprouting") and the subsequent formation of complex anastomosing networks (Korff and Augustin, 1999, J Cell Sci 112:3249-58). In an exemplary experimental set-up, spheroids are prepared by pipetting 400 human umbilical vein endothelial cells into individual wells of a nonadhesive 96-well plates to allow overnight spheroidal aggregation (Korff and Augustin: J Cell Biol 143: 1341-52, 1998). Spheroids are harvested and seeded in 900 μ l of methocel-collagen solution and pipetted into individual wells of a 24 well plate to allow collagen gel polymerization. Test agents

are added after 30 min by pipetting 100 μ l of 10-fold concentrated working dilution of the test substances on top of the gel. Plates are incubated at 37°C for 24h. Dishes are fixed at the end of the experimental incubation period by addition of paraformaldehyde. Sprouting intensity of endothelial cells can be quantitated by an automated image analysis system to determine the cumulative sprout length per spheroid.

Primary assays for antibody modulators

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the MBCAT protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999, *supra*). The enzyme-linked immunosorbant assay (ELISA) is a preferred method for detecting MBCAT-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

In some cases, screening assays described for small molecule modulators may also be used to test antibody modulators.

Primary assays for nucleic acid modulators

For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance MBCAT gene expression, preferably mRNA expression. In general, expression analysis comprises comparing MBCAT expression in like populations of cells (e.g., two pools of cells that endogenously or recombinantly express MBCAT) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (e.g., using the TaqMan®, PE Applied Biosystems), or microarray analysis may be used to confirm that MBCAT mRNA expression is reduced in cells treated with the nucleic acid modulator (e.g., Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, eds., John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either

the MBCAT protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, *supra*).

In some cases, screening assays described for small molecule modulators, particularly in assay systems that involve MBCAT mRNA expression, may also be used to test nucleic acid modulators.

Secondary Assays

Secondary assays may be used to further assess the activity of MBCAT-modulating agent identified by any of the above methods to confirm that the modulating agent affects MBCAT in a manner relevant to the beta-catenin pathway. As used herein, MBCAT-modulating agents encompass candidate clinical compounds or other agents derived from previously identified modulating agent. Secondary assays can also be used to test the activity of a modulating agent on a particular genetic or biochemical pathway or to test the specificity of the modulating agent's interaction with MBCAT.

Secondary assays generally compare like populations of cells or animals (e.g., two pools of cells or animals that endogenously or recombinantly express MBCAT) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate MBCAT-modulating agent results in changes in the beta-catenin pathway in comparison to untreated (or mock- or placebo-treated) cells or animals. Certain assays use "sensitized genetic backgrounds", which, as used herein, describe cells or animals engineered for altered expression of genes in the beta-catenin or interacting pathways.

Cell-based assays

Cell based assays may detect endogenous beta-catenin pathway activity or may rely on recombinant expression of beta-catenin pathway components. Any of the aforementioned assays may be used in this cell-based format. Candidate modulators are typically added to the cell media but may also be injected into cells or delivered by any other efficacious means.

Animal Assays

A variety of non-human animal models of normal or defective beta-catenin pathway may be used to test candidate MBCAT modulators. Models for defective beta-catenin pathway typically use genetically modified animals that have been engineered to mis-express (e.g., over-express or lack expression in) genes involved in the beta-catenin pathway. Assays generally require systemic delivery of the candidate modulators, such as by oral administration, injection, etc.

In a preferred embodiment, beta-catenin pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal beta-catenin are used to test the candidate modulator's affect on MBCAT in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4°C, but rapidly forms a solid gel at 37°C. Liquid Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the MBCAT. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with Matrigel® pellets may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5 - 12 days post-injection, and the Matrigel® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.

In another preferred embodiment, the effect of the candidate modulator on MBCAT is assessed via tumorigenicity assays. Tumor xenograft assays are known in the art (see, e.g., Ogawa K et al., 2000, *Oncogene* 19:6043-6052). Xenografts are typically implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from *in vitro* culture. The tumors which express the MBCAT endogenously are injected in the flank, 1×10^5 to 1×10^7 cells per mouse in a volume of 100 μ L using a 27gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is

assessed by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors maybe utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4% paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4°C, immersed in 30% sucrose in PBS, and rapidly frozen in isopentane cooled with liquid nitrogen.

In another preferred embodiment, tumorogenicity is monitored using a hollow fiber assay, which is described in U.S. Pat No. US 5,698,413. Briefly, the method comprises implanting into a laboratory animal a biocompatible, semi-permeable encapsulation device containing target cells, treating the laboratory animal with a candidate modulating agent, and evaluating the target cells for reaction to the candidate modulator. Implanted cells are generally human cells from a pre-existing tumor or a tumor cell line. After an appropriate period of time, generally around six days, the implanted samples are harvested for evaluation of the candidate modulator.

Tumorogenicity and modulator efficacy may be evaluated by assaying the quantity of viable cells present in the macrocapsule, which can be determined by tests known in the art, for example, MTT dye conversion assay, neutral red dye uptake, trypan blue staining, viable cell counts, the number of colonies formed in soft agar, the capacity of the cells to recover and replicate in vitro, etc.

In another preferred embodiment, a tumorogenicity assay use a transgenic animal, usually a mouse, carrying a dominant oncogene or tumor suppressor gene knockout under the control of tissue specific regulatory sequences; these assays are generally referred to as transgenic tumor assays. In a preferred application, tumor development in the transgenic model is well characterized or is controlled. In an exemplary model, the "RIP1-Tag2" transgene, comprising the SV40 large T-antigen oncogene under control of the insulin gene regulatory regions is expressed in pancreatic beta cells and results in islet cell carcinomas (Hanahan D, 1985, Nature 315:115-122; Parangi S et al, 1996, Proc Natl Acad Sci USA 93: 2002-2007; Bergers G et al, 1999, Science 284:808-812). An "angiogenic switch," occurs at approximately five weeks, as normally quiescent capillaries in a subset of hyperproliferative islets become angiogenic. The RIP1-TAG2 mice die by age 14 weeks. Candidate modulators may be administered

at a variety of stages, including just prior to the angiogenic switch (e.g., for a model of tumor prevention), during the growth of small tumors (e.g., for a model of intervention), or during the growth of large and/or invasive tumors (e.g., for a model of regression). Tumorigenicity and modulator efficacy can be evaluated life-span extension and/or tumor characteristics, including number of tumors, tumor size, tumor morphology, vessel density, apoptotic index, etc.

Diagnostic and therapeutic uses

Specific MBCAT-modulating agents are useful in a variety of diagnostic and therapeutic applications where disease or disease prognosis is related to defects in the beta-catenin pathway, such as angiogenic, apoptotic, or cell proliferation disorders. Accordingly, the invention also provides methods for modulating the beta-catenin pathway in a cell, preferably a cell pre-determined to have defective or impaired beta-catenin function (e.g. due to overexpression, underexpression, or misexpression of beta-catenin, or due to gene mutations), comprising the step of administering an agent to the cell that specifically modulates MBCAT activity. Preferably, the modulating agent produces a detectable phenotypic change in the cell indicating that the beta-catenin function is restored. The phrase "function is restored", and equivalents, as used herein, means that the desired phenotype is achieved, or is brought closer to normal compared to untreated cells. For example, with restored beta-catenin function, cell proliferation and/or progression through cell cycle may normalize, or be brought closer to normal relative to untreated cells. The invention also provides methods for treating disorders or disease associated with impaired beta-catenin function by administering a therapeutically effective amount of an MBCAT -modulating agent that modulates the beta-catenin pathway. The invention further provides methods for modulating MBCAT function in a cell, preferably a cell pre-determined to have defective or impaired MBCAT function, by administering an MBCAT -modulating agent. Additionally, the invention provides a method for treating disorders or disease associated with impaired MBCAT function by administering a therapeutically effective amount of an MBCAT -modulating agent.

The discovery that MBCAT is implicated in beta-catenin pathway provides for a variety of methods that can be employed for the diagnostic and prognostic evaluation of

diseases and disorders involving defects in the beta-catenin pathway and for the identification of subjects having a predisposition to such diseases and disorders.

Various expression analysis methods can be used to diagnose whether MBCAT expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR, and microarray analysis. (e.g., Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, eds., John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm and Guiseppi-Elie, Curr Opin Biotechnol 2001, 12:41-47). Tissues having a disease or disorder implicating defective beta-catenin signaling that express an MBCAT, are identified as amenable to treatment with an MBCAT modulating agent. In a preferred application, the beta-catenin defective tissue overexpresses an MBCAT relative to normal tissue. For example, a Northern blot analysis of mRNA from tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial MBCAT cDNA sequences as probes, can determine whether particular tumors express or overexpress MBCAT. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of MBCAT expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

Various other diagnostic methods may be performed, for example, utilizing reagents such as the MBCAT oligonucleotides, and antibodies directed against an MBCAT, as described above for: (1) the detection of the presence of MBCAT gene mutations, or the detection of either over- or under-expression of MBCAT mRNA relative to the non-disorder state; (2) the detection of either an over- or an under-abundance of MBCAT gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by MBCAT.

Thus, in a specific embodiment, the invention is drawn to a method for diagnosing a disease or disorder in a patient that is associated with alterations in MBCAT expression, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for MBCAT expression; c) comparing results from step (b) with a control; and d) determining whether step (c) indicates a likelihood of the disease or disorder. Preferably, the disease is cancer. The probe may be either DNA or protein, including an antibody.

EXAMPLES

The following experimental section and examples are offered by way of illustration and not by way of limitation.

I. Drosophila beta-catenin screen

Two dominant loss of function screens were carried out in *Drosophila* to identify genes that interact with the Wg cell signaling molecule, beta -catenin (Riggleman et al. (1990) Cell 63:549-560; Peifer et al. (1991) Development 111:1029-1043). Late stage activation of the pathway in the developing *Drosophila* eye leads to apoptosis (Freeman and Bienz (2001) EMBO reports 2: 157-162), whereas early stage activation leads to an overgrowth phenotype. We discovered that ectopic expression of the activated protein in the wing results in changes of cell fate into ectopic bristles and wing veins.

Each transgene was carried in a separate fly stock:

Stocks and genotypes were as follows:

eye overgrowth transgene: isow; P{3.5 eyeless-Gal4}; P{arm(S56F)-pExp-UAS})/TM6b;

eye apoptosis transgene: y w; P{arm(S56F)-pExp-GMR}/CyO; and

wing transgene: P{arm(ΔN)-pExp-VgMQ}/FM7c

In the first dominant loss of function screen, females of each of these three transgenes were crossed to a collection of males containing genomic deficiencies. Resulting progeny containing the transgene and the deficiency were then scored for the effect of the deficiency on the eye apoptosis, eye overgrowth, and wing phenotypes, i.e., whether the deficiency enhanced, suppressed, or had no effect on their respective phenotypes. All data was recorded and all modifiers were retested with a repeat of the original cross. Modifying deficiencies of the phenotypes were then prioritized according to how they modified each of the three phenotypes.

Transposons contained within the prioritized deficiencies were then screened as described. Females of each of the three transgenes were crossed to a collection of 4 types of transposons (3 piggyBac-based and 1 P-element-based). The resulting progeny containing the transgene and the transposon were scored for the effect of the transposon.

on their respective phenotypes. All data was recorded and all modifiers were retested with a repeat of the original cross. Modifiers of the phenotypes were identified as either members of the Wg pathway, components of apoptotic related pathways, components of cell cycle related pathways, or cell adhesion related proteins.

In the second dominant loss of function screen, females of the eye overgrowth transgene were crossed to males from a collection of 3 types of piggyBac-based transposons. The resulting progeny containing the transgene and the transposon were scored for the effect of the transposon on the eye overgrowth phenotype. All data was recorded and all modifiers were retested with a repeat of the original cross. Modifiers of the phenotypes were identified as either members of the Wg pathway, components of cell cycle related pathways, or cell adhesion related proteins.

II. Analysis of Table 1

BLAST analysis (Altschul et al., *supra*) was employed to identify orthologs of *Drosophila* modifiers. The columns "MBCAT symbol", and "MBCAT name aliases" provide a symbol and the known name abbreviations for the Targets, where available, from Genbank. "MBCAT RefSeq_NA or GI_NA", "MBCAT GI_AA", "MBCAT NAME", and "MBCAT Description" provide the reference DNA sequences for the MBCATs as available from National Center for Biology Information (NCBI), MBCAT protein Genbank identifier number (GI#), MBCAT name, and MBCAT description, all available from Genbank, respectively. The length of each amino acid is in the "MBCAT Protein Length" column.

Names and Protein sequences of *Drosophila* modifiers of beta-catenin from screen (Example I), are represented in the "Modifier Name" and "Modifier GI_AA" column by GI#, respectively.

Table 1

MBCAT symbol	MBCAT name aliases	MBCAT RefSeq_NA or GI_NA	MBCAT T GI_AA	MBCAT name	MBCAT description	MBCAT protein length	Modifier name	Modifier gi_aa

EX03-053P

ADCY5	ADCY5 adenylyl cyclase 5 adenylyl cyclase type V adenylyl cyclase type 5	XM_171048	22212711	adenylate cyclase 5	response to hypoxia	894	CG32158	24665354
ADCY6	KIAA0422 adenylyl cyclase 6 ADCY6 adenylyl cyclase type VI	NM_015270 NM_02960983	101810	adenylate cyclase 6	regulation of smooth muscle contraction; cAMP biosynthesis	1168	CG32158	24665354
ADCY8	ADCY8 ADCY3 HBAC1 Adenylyl cyclase-8, brain adenylyl cyclase 8 (brain) adenylyl cyclase type VIII	NM_001115	4557257	adenylate cyclase 8 (brain)	learning and/or memory; response to drug; signal transduction	1251	CG32158	24665354
CENTG1	CENTG1 PIKE AGAP2 GGAP2 KIAA0167 centaurin gamma 1 phosphoinositide 3-kinase enhancer GTP-binding and GTPase activating protein 2 Arf GAP with GTP-binding protein-like, ANK repeat and PH domains 2 centaurin, gamma 1	NM_014770 NM_023017	766196	centaurin, gamma 1	na	836	cenG1A	24584217
CENTG2	CENTG2 AGAP1 GGAP1 KIAA1099 GTP-binding and GTPase-activating protein 1 Arf GAP with GTP-binding protein-like, ANK repeat and PH domains 1 centaurin, gamma 2	NM_014914	7662484	centaurin, gamma 2	vesicle-mediated transport; actin cytoskeleton organization and biogenesis	804	cenG1A	24584217
CENTG3	CENTG3 MRIP-1 MRIP-1 protein centaurin, gamma 3	NM_031946	16799069	centaurin, gamma 3	cell growth and/or maintenance; signal transduction; signal transduction	875	cenG1A	24584217

EX03-053P

DDX17	DDX17 P72 RH70 probable RNA-dependent helicase p72 DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 17 (72kD) DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 17, 72kDa DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	NM_006386 NM_030881	545384	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	regulation of transcription from Pol II promoter; RNA processing	650	Rm62	24644481	
DDX5	DDX5 P68 HLR1 G17P1 HUMP68 RNA-dependent ATPase DEAD/H box-5 (RNA helicase, 68kD) DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68kD) DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68kDa) p68 DEAD box-5 DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	NM_004396	4758138	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	mRNA splicing	614	Rm62	24644481	
FLJ10665	FLJ10665 hypothetical protein FLJ10665	NM_018173	8922581	hypothetical protein FLJ10665	Rho protein signal transduction; cell growth and/or maintenance	790	CG7323	7293694	
KIAA0720	KIAA0720 KIAA0720 protein	XM_030970	29728516	KIAA0720 protein	Rho protein signal transduction; cell growth and/or maintenance	1083	CG7323	7293694	
FLJ20530	FLJ20530 hypothetical protein FLJ20530	NM_017864	8923496	hypothetical protein FLJ20530	na	557	EG:EG003.5	18484916	

EX03-053P

GABRA2	GABRA2 gamma-aminobutyric acid (GABA) A receptor, alpha 2	NM_000807	4557601	gamma-aminobutyric acid (GABA) A receptor, alpha 2	gamma-amino butyric acid signaling pathway; gamma-amino butyric acid signaling pathway; chloride transport; regulation of neurotransmitter levels	451	GluClap ha	24648249
GABRA5	GABRA5 gamma-aminobutyric acid (GABA) A receptor, alpha 5	NM_000810	4503861	gamma-aminobutyric acid (GABA) A receptor, alpha 5	gamma-amino butyric acid signaling pathway; chloride transport; synaptic transmission; signal transduction	462	GluClap ha	24648249
GLRA1	GLRA1 STHE glycine receptor, alpha 1 (startle disease/hyperekplexia, stiff man syndrome)	NM_000171	4504019	glycine receptor, alpha 1 (startle disease/hyperekplexia, stiff man syndrome)	cell surface receptor linked signal transduction; neurogenesis; chloride transport; chloride transport; synaptic transmission; synaptic transmission; synaptic transmission	449	GluClap ha	24648249
GYG	GYG glycogenin	NM_004130	20127457	glycogenin	glycogen biosynthesis; glycogen biosynthesis; glycogen biosynthesis	350	Glycogen in	24656813
GYG2	GYG2 GN2 GN-2 glycogenin 2	NM_003918	5453674	glycogenin 2	glycogen biosynthesis; glycogen biosynthesis	501	Glycogen in	24656813
LOC350705	LOC350705 similar to Glycogenin-1	XM_301113	30147855	similar to Glycogenin-1	na	233	Glycogen in	24656813

EX03-053P

MLLT10	MLLT10 AF10 ALL1 fused gene from chromosome 10 myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 10 myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	NM_004641	4757726	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10	na	1027	Alhambra	24644741	
MLLT6	MLLT6 AF17 Myeloid/lymphoid or mixed-lineage leukemia, translocated to, 6 myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 6 myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 6	NM_005937	5174577	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 6	cell growth and/or maintenance; regulation of transcription, DNA-dependent	1093	Alhambra	24644741	
POU2F1	POU2F1 OCT1 OTF1 Octamer-binding transcription factor-1 POU domain, class 2, transcription factor 1	NM_002697	4505957	POU domain, class 2, transcription factor 1	transcription from Pol II promoter; regulation of transcription from Pol II promoter; negative regulation of transcription; regulation of transcription from Pol III promoter	743	pdm2	24583942	
POU2F2	POU2F2 OCT2 OTF2 POU domain, class 2, transcription factor 2	NM_002698	4505959	POU domain, class 2, transcription factor 2	transcription from Pol II promoter; regulation of transcription, DNA-dependent; humoral immune response; membrane fusion; pathogenesis; development	463	pdm2	24583942	

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POU2F3	POU2F3 OCT11 PLA-1 Epoch-1 Skn-1a POU transcription factor 3 likely ortholog of mouse POU domain, class 2, transcription factor 3 POU domain, class 2, transcription factor 3	NM_014352	7657409	POU domain, class 2, transcription factor 3	keratinocyte differentiation; epidermal differentiation; regulation of transcription from Pol II promoter	436	pdm2	24583942
PRKCI	PKCI DDX1179E protein kinase C, iota PRKCI PKCi	NM_002740	4506071	protein kinase C, iota	anti-apoptosis; signal transduction; signal transduction; protein amino acid phosphorylation	587	aPKC	24653760

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PRKCZ	protein kinase C, zeta PRKCZ PKC2 PKCz zeta	NM_002744	10864650	protein kinase C, zeta	insulin receptor signaling pathway; cytokinesis; glucose transport; antibacterial humoral response (sensu Vertebrata); activation of MAP/ERK kinase kinase; positive regulation of transcription from Pol II promoter; actin cytoskeleton organization and biogenesis; T-cell activation; protein kinase cascade; anti-apoptosis; chemotaxis; positive regulation of cell proliferation; monocyte activation; cytokine biosynthesis; cytokine and chemokine mediated signaling pathway; cell cycle arrest; signal transduction; signal transduction; signal transduction; inflammatory response; protein amino acid phosphorylation; protein amino acid phosphorylation; protein amino acid phosphorylation; protein amino acid phosphorylation	592	aPKC	24653760	
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SLC1A2	SLC1A2 EAAT2 GLT-1 glutamate/aspartate transporter II excitatory amino acid transporter 2 H.sapiens mRNA for glutamate transporter glial high affinity glutamate transporter solute carrier family 1 (glial high affinity glutamate transporter), member 2	NM_004171	4759124	solute carrier family 1 (glial high affinity glutamate transporter), member 2	neurotransmitter uptake; synaptic transmission; glutamate transport; glutamate transport	574	Eaat2	17137666
SLC1A3	SLC1A3 EAAT1 GLAST GLAST1 solute carrier family 1 (glial high affinity glutamate transporter), member 3	NM_004172	4759126	solute carrier family 1 (glial high affinity glutamate transporter), member 3	neurotransmitter uptake; neurotransmitter uptake; neurotransmitter uptake; synaptic transmission; glutamate transport; glutamate transport	542	Eaat2	17137666
SLC1A7	SLC1A7 AAAT EAAT5 excitatory amino acid transporter 5 (retinal glutamate transporter) solute carrier family 1 (glutamate transporter), member 7	NM_006671	20070239	solute carrier family 1 (glutamate transporter), member 7	vision; glutamate transport	560	Eaat2	17137666
SLC1A4	SLC1A4 SATT ASCT1 glutamate/neutral amino acid transporter alanine-serine/cysteine/threonine transporter solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	NM_003038	21314632	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	neutral amino acid transport; neutral amino acid transport	532	Eaat2	17137666

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SLC1A6	SLC1A6 EAAT4 excitatory amino acid transporter 4 solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	NM_005071	4827012	solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6	aspartate transport; chloride transport; synaptic transmission; glutamate transport; glutamate transport; glutamate transport	564	Eaat2	17137666
SLC1A1	SLC1A1 EAAC1 EAAT3 excitatory amino acid carrier 1 excitatory amino acid transporter-3 solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	NM_004170	18252049	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1	synaptic transmission; glutamate transport	524	Eaat2	17137666
SLC1A5	SLC1A5 R16 AAAT ATBO M7V1 RDRC ASCT2 M7VS1 RD114 virus receptor baboon M7 virus receptor neutral amino acid transporter B solute carrier family 1 (neutral amino acid transporter), member 5	NM_005628	5032093	solute carrier family 1 (neutral amino acid transporter), member 5	neutral amino acid transport; neutral amino acid transport; EGF receptor signaling pathway	541	Eaat2	17137666
SLC7A1	SLC7A1 ERR ATRC1 CAT-1 HCAT1 RECIL ecotropic retroviral receptor amino acid transporter, cationic 1 solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 1	NM_003045	4507047	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 1	basic amino acid transport; amino acid transport; arginine transport; amino acid metabolism	629	CG11128	7296600

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SLC7A2	SLC7A2 ATRC2 CAT-2 HCAT2 amino acid transporter, cationic 2 solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 2	NM_003046	4507049	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 2	basic amino acid transport; amino acid metabolism	657	CG11128	7296600
SLC7A3	SLC7A3 ATRC3 CAT-3 FLJ14541 solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 3	NM_032803	17939406	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 3	amino acid transport; amino acid transport; amino acid metabolism; L-amino acid transport; L-amino acid transport	619	CG11128	7296600
SULF2	SULF2 SULF-2 HSULF-2 KIAA1247 sulfatase 2 similar to glucosamine-6-sulfatases	NM_018837 XM_030036	29789100	similar to glucosamine-6-sulfatases	metabolism	870	Sulf1	18487383
SULF1	SULF1 SULF-1 HSULF-1 KIAA1077 sulfatase FP sulfatase 1	NM_015170	29789064	sulfatase 1	metabolism	871	Sulf1	18487383
LOC151531	LOC151531 liver-specific uridine phosphorylase	NM_173355	27597096	liver-specific uridine phosphorylase	na	317	CG6330	23172399
UP	UP uridine phosphorylase	NM_003364 NM_181597	4507839	uridine phosphorylase	nucleobase, nucleoside, nucleotide and nucleic acid metabolism; response to drug; response to drug; response to drug; response to drug	310	CG6330	23172399

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RAB32	RAB32 RAB32, member RAS oncogene family	NM_006834	580313	RAB32, member RAS oncogene family	small GTPase mediated signal transduction; organelle organization and biogenesis; protein-vacuolar targeting; exocytosis; endocytosis; mitochondrial membrane organization and biogenesis; membrane fusion	225	Rab-RP1	6561893
RAB38	RAB38 NY-MEL-1 Rab-related GTP-binding protein RAB38, member RAS oncogene family	NM_022337	116412	RAB38, member RAS oncogene family	small GTPase mediated signal transduction; vesicle-mediated transport	211	Rab-RP1	6561893
RAB7L1	RAB7L1 RAB7, member RAS oncogene family-like 1	NM_003929	450637	RAB7, member RAS oncogene family-like 1	na	203	Rab-RP1	6561893
C6orf37	C6orf37 FLJ20037 retinal expressed gene C6orf37 chromosome 6 open reading frame 37	NM_017633	892304	chromosome 6 open reading frame 37	na	447	CG30497	24586361
FLJ20202	FLJ20202 hypothetical protein FLJ20202	NM_017709	892319	hypothetical protein FLJ20202	na	391	CG30497	24586361
MGC16491	MGC16491 MGC20845 hypothetical protein MGC16491	NM_052943	164184	hypothetical protein MGC16491	na	424	CG30497	24586361
MGC26999	MGC26999 hypothetical protein MGC26999	NM_152630	227492	hypothetical protein MGC26999	na	389	CG30497	24586361

III. High-Throughput In Vitro Fluorescence Polarization Assay

Fluorescently-labeled MBCAT peptide/substrate are added to each well of a 96-well microtiter plate, along with a test agent in a test buffer (10 mM HEPES, 10 mM NaCl, 6 mM magnesium chloride, pH 7.6). Changes in fluorescence polarization, determined by using a Fluorolite FPM-2 Fluorescence Polarization Microtiter System (Dynatech Laboratories, Inc), relative to control values indicates the test compound is a candidate modifier of MBCAT activity.

IV. High-Throughput In Vitro Binding Assay.

^{33}P -labeled MBCAT peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH 7.6, 1 mM MgCl₂, 1% glycerol, 0.5% NP-40, 50 mM beta-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors) along with a test agent to the wells of a Neutralite-avidin coated assay plate and incubated at 25°C for 1 hour. Biotinylated substrate is then added to each well and incubated for 1 hour. Reactions are stopped by washing with PBS, and counted in a scintillation counter. Test agents that cause a difference in activity relative to control without test agent are identified as candidate beta-catenin modulating agents.

V. Immunoprecipitations and Immunoblotting

For coprecipitation of transfected proteins, 3×10^6 appropriate recombinant cells containing the MBCAT proteins are plated on 10-cm dishes and transfected on the following day with expression constructs. The total amount of DNA is kept constant in each transfection by adding empty vector. After 24 h, cells are collected, washed once with phosphate-buffered saline and lysed for 20 min on ice in 1 ml of lysis buffer containing 50 mM Hepes, pH 7.9, 250 mM NaCl, 20 mM -glycerophosphate, 1 mM sodium orthovanadate, 5 mM p-nitrophenyl phosphate, 2 mM dithiothreitol, protease inhibitors (complete, Roche Molecular Biochemicals), and 1% Nonidet P-40. Cellular debris is removed by centrifugation twice at 15,000 $\times g$ for 15 min. The cell lysate is incubated with 25 μl of M2 beads (Sigma) for 2 h at 4 °C with gentle rocking.

After extensive washing with lysis buffer, proteins bound to the beads are solubilized by boiling in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane and blotted with the indicated antibodies. The reactive bands are visualized with horseradish peroxidase coupled to the appropriate secondary antibodies and the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham Pharmacia Biotech).

VI. Kinase assay

A purified or partially purified MBCAT is diluted in a suitable reaction buffer, e.g., 50 mM Hepes, pH 7.5, containing magnesium chloride or manganese chloride (1-20 mM) and a peptide or polypeptide substrate, such as myelin basic protein or casein (1-10 μ g/ml). The final concentration of the kinase is 1-20 nM. The enzyme reaction is conducted in microtiter plates to facilitate optimization of reaction conditions by increasing assay throughput. A 96-well microtiter plate is employed using a final volume 30-100 μ l. The reaction is initiated by the addition of 33 P-gamma-ATP (0.5 μ Ci/ml) and incubated for 0.5 to 3 hours at room temperature. Negative controls are provided by the addition of EDTA, which chelates the divalent cation (Mg^{2+} or Mn^{2+}) required for enzymatic activity. Following the incubation, the enzyme reaction is quenched using EDTA. Samples of the reaction are transferred to a 96-well glass fiber filter plate (MultiScreen, Millipore). The filters are subsequently washed with phosphate-buffered saline, dilute phosphoric acid (0.5%) or other suitable medium to remove excess radiolabeled ATP. Scintillation cocktail is added to the filter plate and the incorporated radioactivity is quantitated by scintillation counting (Wallac/Perkin Elmer). Activity is defined by the amount of radioactivity detected following subtraction of the negative control reaction value (EDTA quench).

VII. Expression analysis

All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC (American Type Culture Collection, Manassas, VA 20110-2209). Normal and tumor tissues are obtained from Impath, UC Davis, Clontech, Stratagene, Ardais, Genome Collaborative, and Ambion.

TaqMan analysis is used to assess expression levels of the disclosed genes in various samples.

RNA is extracted from each tissue sample using Qiagen (Valencia, CA) RNeasy kits, following manufacturer's protocols, to a final concentration of 50ng/ μ l. Single stranded cDNA is then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems (Foster City, CA).

Primers for expression analysis using TaqMan assay (Applied Biosystems, Foster City, CA) are prepared according to the TaqMan protocols, and the following criteria: a) primer pairs are designed to span introns to eliminate genomic contamination, and b) each primer pair produced only one product. Expression analysis is performed using a 7900HT instrument.

Taqman reactions are carried out following manufacturer's protocols, in 25 μ l total volume for 96-well plates and 10 μ l total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis is prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts is good. The raw data are normalized using 18S rRNA (universally expressed in all tissues and cells).

For each expression analysis, tumor tissue samples are compared with matched normal tissues from the same patient. A gene is considered overexpressed in a tumor when the level of expression of the gene is 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue is not available, a universal pool of cDNA samples is used instead. In these cases, a gene is considered overexpressed in a tumor sample when the difference of expression levels between a tumor sample and the average of all normal samples from the same tissue type is greater than 2 times the standard deviation of all normal samples (i.e., $\text{Tumor} - \text{average(all normal samples)} > 2 \times \text{STDEV(all normal samples)}$).

A modulator identified by an assay described herein can be further validated for therapeutic effect by administration to a tumor in which the gene is overexpressed. A decrease in tumor growth confirms therapeutic utility of the modulator. Prior to treating

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a patient with the modulator, the likelihood that the patient will respond to treatment can be diagnosed by obtaining a tumor sample from the patient, and assaying for expression of the gene targeted by the modulator. The expression data for the gene(s) can also be used as a diagnostic marker for disease progression. The assay can be performed by expression analysis as described above, by antibody directed to the gene target, or by any other available detection method.

WHAT IS CLAIMED IS:

1. A method of identifying a candidate beta-catenin pathway modulating agent, said method comprising the steps of:
 - (a) providing an assay system comprising a MBCAT polypeptide or nucleic acid;
 - (b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and
 - (c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as a candidate beta-catenin pathway modulating agent.
2. The method of Claim 1 wherein the assay system comprises cultured cells that express the MBCAT polypeptide.
3. The method of Claim 2 wherein the cultured cells additionally have defective beta-catenin function.
4. The method of Claim 1 wherein the assay system includes a screening assay comprising a MBCAT polypeptide, and the candidate test agent is a small molecule modulator.
5. The method of Claim 4 wherein the assay is a binding assay.
6. The method of Claim 1 wherein the assay system is selected from the group consisting of an apoptosis assay system, a cell proliferation assay system, an angiogenesis assay system, and a hypoxic induction assay system.
7. The method of Claim 1 wherein the assay system includes a binding assay comprising a MBCAT polypeptide and the candidate test agent is an antibody.

8. The method of Claim 1 wherein the assay system includes an expression assay comprising a MBCAT nucleic acid and the candidate test agent is a nucleic acid modulator.

9. The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.

10. The method of Claim 8 wherein the nucleic acid modulator is a PMO.

11. The method of Claim 1 additionally comprising:

(d) administering the candidate beta-catenin pathway modulating agent identified in (c) to a model system comprising cells defective in beta-catenin function and, detecting a phenotypic change in the model system that indicates that the beta-catenin function is restored.

12. The method of Claim 11 wherein the model system is a mouse model with defective beta-catenin function.

13. A method for modulating a beta-catenin pathway of a cell comprising contacting a cell defective in beta-catenin function with a candidate modulator that specifically binds to a MBCAT polypeptide, whereby beta-catenin function is restored.

14. The method of claim 13 wherein the candidate modulator is administered to a vertebrate animal predetermined to have a disease or disorder resulting from a defect in beta-catenin function.

15. The method of Claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.

16. The method of Claim 1, comprising the additional steps of:

(d) providing a secondary assay system comprising cultured cells or a non-human animal expressing MBCAT ,

(e) contacting the secondary assay system with the test agent of (b) or an agent derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and

(f) detecting an agent-biased activity of the second assay system, wherein a difference between the agent-biased activity and the reference activity of the second assay system confirms the test agent or agent derived therefrom as a candidate beta-catenin pathway modulating agent,

and wherein the second assay detects an agent-biased change in the beta-catenin pathway.

17. The method of Claim 16 wherein the secondary assay system comprises cultured cells.

18. The method of Claim 16 wherein the secondary assay system comprises a non-human animal.

19. The method of Claim 18 wherein the non-human animal mis-expresses a beta-catenin pathway gene.

20. A method of modulating beta-catenin pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds a MBCAT polypeptide or nucleic acid.

21. The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the beta-catenin pathway.

22. The method of Claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.

23. A method for diagnosing a disease in a patient comprising:

- (a) obtaining a biological sample from the patient;
- (b) contacting the sample with a probe for MBCAT expression;
- (c) comparing results from step (b) with a control;
- (d) determining whether step (c) indicates a likelihood of disease.

24. The method of claim 23 wherein said disease is cancer.

ABSTRACT OF THE DISCLOSURE

Human MBCAT genes are identified as modulators of the beta-catenin pathway, and thus are therapeutic targets for disorders associated with defective beta-catenin function. Methods for identifying modulators of beta-catenin, comprising screening for agents that modulate the activity of MBCAT are provided.

Nucleic Acid and Polypeptide sequences

>gi|27480832|ref|XM_171048.2| Homo sapiens adenylate cyclase 5 (ADCY5).

mRNA

ACATCGTGGGTGCTGCACCCACTATCCGGCTGAGGTCTCCAGAGACAGGTTCCAGGAGACCCGAGA
 GTGCATCCAGCGCGGCTCACTCGCAGCGGGAGAACAGCAGCAGGAACGGCTCTGCTGTCTGCCTT
 CCCGTATGTTGCATGGAGATGAAAGCAGACATCAACGCCAAGCAGGAGGATATGATGTTCCATAAGA
 TTTACATCCAGAAACATGACAACGTGAGCATCCTGTTGTCACATCGAGGGCTGAGGGGCCCTCGACTC
 AGCTGGGGTAGACGGGCTCGAATGTGGCCTGGGAGAGCTAGGGGGCCCAAGGGCTGCTTTCTATG
 TGAGCCTTAAACCTCAGACAGGCCACCCCTGCACCTGCAGGGCTTGGCACAGGAGTGCCTGGCTT
 GGAGGGACTGTGGGCTTCATCGTGGCTCTGCCCACACCTCACGCACACAGACAGTGCCTAGGAGG
 AAACAGAACTAATTACGAGGGGAGGCAAGAGGACGCCAGGAAGGAGTGTGATCTGAGAAAAATATT
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>gi|10947059|ref|NM_015270.2| Homo sapiens adenylate cyclase 6 (ADCY6). transcript variant 1, mRNA

CCGGTCTATAGGTGCATACCACCAACACCAGGCTAATTTCAGGGATCTCAGTCAACCTCTGCCACCCGGGTTCAAGCAATTCTCC
 CCAGGCTGGAGTGCAATGGCGGATCTTGTCACTGCAACCTCTGCCACCCGGGTTCAAGCAATTCTCC
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>gi|4557256|ref|NM_001115.1| Homo sapiens adenylyl cyclase 8 (brain)
(ADCY8), mRNA
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>gi|29728515|ref|XM_030970.9| Homo sapiens KIAA0720 protein (KIAA0720).
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>gi|8923495|ref|NM_017864.1| Homo sapiens hypothetical protein FLJ20530
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>gi|6031207|ref|NM_000810.2| Homo sapiens gamma-aminobutyric acid
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>gi|4504018|ref|NM_000171.1| Homo sapiens glycine receptor, alpha 1
 (startle disease/hyperekplexia, stiff man syndrome) (GLRA1), mRNA
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>gi|30147854|ref|XM_301113.1| Homo sapiens similar to Glycogenin-1
 (LOC350705), mRNA
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AG

>gi|4757725|ref|NM_004641.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10 (MLLT10), mRNA

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>gi|5174576|ref|NM_005937.1| Homo sapiens myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 6 (MLLT6), mRNA
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>gi|4505956|ref|NM_002697.1| Homo sapiens POU domain, class 2, transcription factor 1 (POU2F1), mRNA

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>gi|4505958|ref|NM_002698.1| Homo sapiens POU domain, class 2,
 transcription factor 2 (POU2F2), mRNA
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>gi|7657408|ref|NM_014352.1| Homo sapiens POU domain, class 2,
 transcription factor 3 (POU2F3), mRNA
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EX03-053P

>gi|31543438|ref|NM_002740.2| Homo sapiens protein kinase C, iota
(PRKCI), mRNA

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>gi|10864649|ref|NM_002744.2| Homo sapiens protein kinase C, zeta
(PRKCZ), mRNA

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>gi|4759123|ref|NM_004171.1| Homo sapiens solute carrier family 1
 (glial high affinity glutamate transporter), member 2 (SLC1A2), nuclear
 gene encoding mitochondrial protein, mRNA
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>gi|31543627|ref|NM_004172.2| Homo sapiens solute carrier family 1
 (glial high affinity glutamate transporter), member 3 (SLC1A3), mRNA
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EX03-053P

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>gi|20070238|ref|NM_006671.2| Homo sapiens solute carrier family 1
(glutamate transporter), member 7 (SLC1A7), mRNA
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>gi|21314631|ref|NM_003038.2| Homo sapiens solute carrier family 1 (glutamate/neutral amino acid transporter), member 4 (SLC1A4), mRNA
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>gi|4827011|ref|NM_005071.1| Homo sapiens solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6 (SLC1A6), mRNA
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>gi|31543625|ref|NM_004170.3| Homo sapiens solute carrier family 1
 (neuronal/epithelial high affinity glutamate transporter, system Xag),
 member 1 (SLC1A1), mRNA
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>gi|5032092|ref|NM_005628.1| Homo sapiens solute carrier family 1
 (neutral amino acid transporter), member 5 (SLC1A5), mRNA
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>gi|4507046|ref|NM_003045.1| Homo sapiens solute carrier family 7 (cationic amino acid transporter, y⁺ system), member 1 (SLC7A1), mRNA
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>gi|4507048|ref|NM_003046.1| Homo sapiens solute carrier family 7 (cationic amino acid transporter, y⁺ system), member 2 (SLC7A2), mRNA
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EX03-053P

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>gi|31543640|ref|NM_032803.3| Homo sapiens solute carrier family 7
(cationic amino acid transporter, γ -system), member 3 (SLC7A3), mRNA
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>gi|29789099|ref|NM_018837.1| Homo sapiens similar to glucosamine-6-
sulfatases (SULF2), mRNA
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>gi|29789063|ref|NM_015170.1| Homo sapiens sulfatase 1 (SULF1), mRNA
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>gi|27597095|ref|NM_173355.1| Homo sapiens uridine phosphorylase-2
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>gi|31742506|ref|NM_003364.2| Homo sapiens uridine phosphorylase (UP), transcript variant 1, mRNA
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>gi|11641236|ref|NM_022337.1| Homo sapiens RAB38, member RAS oncogene family (RAB38), mRNA
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>gi|4506374|ref|NM_003929.1| Homo sapiens RAB7, member RAS oncogene family-like 1 (RAB7L1), mRNA
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>gi|8923041|ref|NM_017633.1| Homo sapiens open reading frame 37 (C6orf37), mRNA

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>gi|8923191|ref|NM_017709.1| Homo sapiens hypothetical protein FLJ20202 (FLJ20202), mRNA

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ACCTCATGATCTTCGCAAGGGTGGTGAACGAGAGCACCGTGTCTCATGGGGCATGAACGCCAGGCAGAC
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GTCACCTGTTACTACCAGCCGGCCCTTACGTCAGTGTGGCAACTCAGCAACTACTACGTTGCCCATC
CTCCAGTCACCTACAGCCAGCCTTACCCCTACCTGGCTGCCCTGTAACTAACCTTGAGACCTGAGGGTTTC
CACAGTGGAAACCCCAATAGGGCTAGGGCTCTCAGGTAGGGAGGCCCTCTAGATGTAGGCATTGG
TTTTAAAGGGAACTCAGCTGATTCTGCTTTTTTTTTCTCTTGTGACCCATTGGAAATGG
TCTACAGTGATCATGAGCCAACCCCTCAAAGGACCGTATTACAGTGCACGTTGGAAAACGCTACAGGA

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AGCATGACCTATCCACATCTTCCAAGATAGACACTAACATGTCATGCCCCAACATTAGCACGTGGGG
TTGAGCTCTGTGCAGTAATCGAGATTGGGAGAATTGGGCAGCGCGTGAGAAGTGCTAAGCTACTTGT
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TGATAATCTCTCTTGTACCAATTAGTCAGGCAGAAAATGGTAAACATGAGGGTGTCTGTGACT
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>gi|16418426|ref|NM_052943.1| Homo sapiens hypothetical protein
MGC16491 (MGC16491), mRNA
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>gi|22749286|ref|NM_152630.1| Homo sapiens hypothetical protein
MGC26999 (MGC26999), mRNA
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AAGCAATCTTCAATTGATCTACTGACTTCACAATGTCGAAATCAGATTCCAAATCTCACTGGGAT
CAAGTTATAACACTGGATCAAGTGTAGATGAAGTAATTCCAATTGAAAGGGAAATTCCCCACAA
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AAAAATAAGTTAAACGAATGAAATGACCAGCTCTGAATGTGAAAGCTTCTATCATCTACTACAA
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GATGTTTATACCAACAGCACAAGTGGCATAGTATTAAAGAATTATATCACAGAACCCCTGAG
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GAAAGGCAAAAAAAAAAAAAAA

>gi|22212711|gb|AAM94374.1|AF497517_1 adenylate cyclase type V [Homo sapiens]
TNAQDQFLKQLVSNLIFSCTNIVGVCTHYPAEVSRQAFQETRECIQARLHSQRENQQERLLLSQLP
RHVAMEMKADINAKQEDMMFHKIYIQLHDNVSILFADIEGFTSLASQCTAQELVMTLNELFARFDKLAAE
NHCLRIKILGDCYCVSLPEARADHAAHCCVEMGMDMIEAISLVRVTGVNVNMRVGIHSGRVHCGVLGL
RKWQFDVWSNDVTLANHMEAGGKAGRIHITKATLNYLNGDYVEPGCCGERNAYLKEHSIETFLIRCTQ
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EFLGRAIDARSIDRLRSEHVRKFLLTFREPDLKKYSKQVDRFGAYVACSLVFLFICFVQITIVPHSI
FMLSFYLTCSLLTLVVFVSIYSCVKLFPSPLOQLTSRKIVRSKMNSTLGVFTITLVFLAFVNMFCTN
SRDLLGCLAQEHNISASQVNACHVAESAVNYSLGDEQFCGSPWPNCNFPEYFTYSVLLSLLACSVFLQI
SCIGKLVLMLAIELIYVLIVEVPVGTLFNDALLVTANAIDFFNNNGTSQCPEHATKVALVUTPIIISVF
VLALYIHLAQQVESTARLDFWLQLQATEEKEEMEELQAYNRRLHNLPKDVAHFLAREERRNDELYYQSC
ECVAVMFASIANFSEFYVELEANNEGVECLLNEIIADFDEIISEDRFRQLEKIKTIGSTYMAASGLND
STYDKVGKTHIKALADFAMKLMQMKYINEHSFNNFQMKIGLNINGPVVAGVIGARKPQYDIWGNTVNVAS
RMDSTGVPDRIQVTTDMYQVLAANTYQLECRGVVKVKGEMMTYFLNGGPPLS

>gi|10181096|ref|NP_056085.1| adenylate cyclase 6 isoform a [Homo sapiens]
MSWFSGLLVPKVDERKTAWGERNGQKRSRRRGTRAGGFCTPRYMSCLRDAEPPSPTPAGPPRCPWQDDAF

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IRRGGPGKGKELGLRAVALGFEDTEVTTAGGTAEVAPDAVPRSGRSCWRRLVQVFQSKQFRSAKLERLY
QRYFFQMNQSSLTLLMAVLVLLTAVLLAFHAAPARPPQPAYVALLACAAALFVGLMVVCNRHSFRQDSMWV
VSYVVLGILAAVQVGGALAADPRSPSAGLWCPFFVYIAYTLLPIRMRAAVLSQLGLSTLHLILAWQLNR
GDAFLWKQLGANVLLFLCTNVIGICHTYPAEVSRQAFQETRGYIQLRHLQHENRQERLLSVPQHV
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LRIKILGDCYYCVSGLPEARADHAHCCVEMGVDIMEAISLREVTVGNVNMVRGIHSGRVHCGVLGLRKW
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AIDARSIDQLRKDHVRRFLLTQFQREDLEKKYSRKVDPRFAYVACALLVFCFCIFCIFQLLIFPHSTLMLGI
YASIFLLLITVILCAVYSCGSLFPKALQRLRSIVRSRAHSTAVGIFSVLLVFTSAIANMFTCNHTPIR
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MFASIANFSEFYVELEANNEGVECLRLNNEIIADFDEIISEERFRQLEKIKTIGSTYMAASGLNASTYDQ
VGRSHITALADYAMRLMEQMKHINEHSFNNFQMKIGLNMGPVAVGIGARKPQYDIWGNTVNSSRMDST
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>gi|4557257|ref|NP_001106.1| adenylyl cyclase 8; Adenylyl cyclase-8, brain [Homo sapiens]
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PAGGGPNHHAPQLSGDSALPLYSLGPGERAHSTCGTKVPERSGSGSASGSGGGDGLGFLHLDCAPNSD
FFLNGGYSYRGVIFPTLRSFKSRDLERLYQRYFLGQRRKSEVMVNLVLTKLTLVLHLSLASAPMDP
LKGILLGFFTGIEVVICALVVVKDTSHTYLQYSGVTVWAMTTQILAAGLGYGLGDGIGYVLFTLFA
TYSMLPLPLTWAILAGLGTSSLQVILQVVIPIRPLAVISINQVVAQAVLFMCMNTAGIFISYLSDRAQRQAF
LETRRCVEARLRETEENQRQERLVLSQLPFRVYLEMINDMTNVEDEHLQHQFHRVIYIHYRENVSLFAD
KGFTNLSTTLSAQELVRMLNELFARFDRLAHEHHCLRIKILGDCYYCVSGLPEPRQDHAHCCVEMGLSM
KTIRYVRSRTKHDVDMRIGIHSGSVLCVGLRKWQFDVWSWDVDIANKLESGGIPGRIHISKATLDCLN
GDYNVEEGHGKERNEFLRKHNIETYLIKQPEDSLLSPEDIVKESVSSSDRRNSGATFTEGWSPELPFD
NIVGKQNTLAALTRNSINLLPNHLLAQALHVQSGPEEINKRIEHTIDLRSGDKLREHIKPFSLMFKDSSL
EHKYSQMRDEVFKSMLVCAFIILFITAIQSLPSSRVMPTIQFSILIMLHSALVLIITTAEDYKCLPLI
LRKTCWCINETYLARNVIIFASILINFLGAILNILWCFDKSIPLKLNLTTFNSAVFTDICSYPEYFVFTG
VLAMVTCAVFLRLNSVLKLAFLIMIAIYALLTETVYAGLFLRYDNLNHSGEDFLGTKEVSLLMAMFLL
AVFYHGQQLEYTARLDFLWRVQAKEEINEMKELREHNNEMLRNILPSHVARHFLEKDRDNEELYSQSYDA
VGVMFASIPGFADFYSQTEMNNQGVECLRLNEIIADFDELLGEDRFQDIEKIKTIGSTYMAVSGLSPEK
QQCEDKWHGLCALADFSALTESIQEINKHSFNNFELRIGISHGSVAVGIGAKPQYDIWGKTVNLASR
MDSTGVSGRIQVPEETYILKDQGFADYRGEIYVKGISEQEGKIKTYFLLGRVQPNPFILPPRRLPGQY
SЛАAVVLGLVQSLNRQRQKQLLNENNNTGIKGHYNRRTLLSPSGTEPGAQAEGTDKSDLP

>gi|7661962|ref|NP_055585.1| centaurin, gamma 1; centaurin gamma 1; phosphoinositide 3-kinase enhancer; Arf GAP with GTP-binding protein-like, ANK repeat and PH domains 2; GTP-binding and GTPase activating protein 2 [Homo sapiens]
MHAQRQFVVAAVRAEVRRHEVAKQALNRLRKLAERVDDPELQDSIQASLDSIREAVINSQEWTLSRSIPE
LRLGVLGDRASGKSSLIHRFLTGSYQVLEKTESEQYKEMLVDGQTHLVLIREEAGAPDAKFGWADAVI
FVFSLEDENSFQAVSRLHGQLSSLRGEGRGLALALVGTQDRISASSPRVVGALARALCADMKRCSYYE
TCATYGLNDRVFQEVAKVVTLRKQQQLAAKSPLSSPSHSAASTPVAGQASNGGHTSDYSSSLPSSF
NVGHRELRAAAAAGLSTPGSLHRAAKRRTSLFANRRGSDSEKRSLSRGETTGSGRAIPIKQSFLLKR
SGNSLNKEWKKYVTLSSNGFLYHPSINDYIHSTHGKEMDLLRTTVKPGKRPRAISAFGPSASINGL
VKDMSTVQMGEGLEATTMPSPSPSSLQPPPDQTSKHLKPDRNLARALSTDCTPSGDLSPSLREPPP
SPMVKKQRRKKLTPSKTEGSAGQAEENFEFLIVSSTGQTWHFEAASFEERDAWVQAIQESQILASLQCC
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>gi|7662484|ref|NP_055729.1| centaurin, gamma 2; Arf GAP with GTP-binding protein-like, ANK repeat and PH domains 1; GTP-binding and GTPase-activating protein 1 [Homo sapiens]
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ELKVGIVGNLASGKSLAVHRYLTGTYVQEESPEGGRFKKEIVVDGQSYLLIRDEGGPPEAQFAMWDAV
IFVFSLEDEISFQTVYHYSRMANRNTSEIPLVLVGTQDAISSANPRVIDDARARKLSNDLKRCYYET
CATYGLNVERVFQDVAQKIVATRKQQLSIGPCKSLPNSPSHSSVCSAQSAVHSQTSNGGSSLSDYSS
SVPSTPSISQKELRIDVPPTANTPTPVRKQSKRRSNTLSRKGSDPDKEKKGLESRADSIGSGRAIPIKQ
GMLLKRSGKSLNKEWKKKVTLCDNGVLTYHPSLHDYMQNVHGKEIDLRTTVKVPGKRPPRTSACAPI
SSPKTNGLSKDMSSLHISPNSDTGLGDSVCSSPSISSTTSPKLDPPPSPHANRKKHRRKSTSNSFKADGL
SGTAAEEQEEFIFIISLTGQTHFEATTYERDAWQAIQESQILASLQCESSKNSRLTSQSEAMALQ
SIRNMRGNSHCVDCETQNPNWASNLGALMCIECSGIHRNLGTHLSRVRSLDDWPIELIKVMSSIGNE
LANSVWEESQGRTPKPSVDSTREEKERWIRAKYEQKLFIAPLPCTELSLGQHLLRATADEDLRTAILLA
HGSRDEVNETCGEGDGRITALHLACRKGNNVLAQLLIWYGVDTARDAGNTALAYQASSQECIDVLLQ
YGCPCDERFVLMATPNLSRRNNNNNNSSGRPTII

>gi|16799069|ref|NP_114152.2| centaurin, gamma 3; MRIP-1 protein [Homo sapiens]
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VSIEDSFVNSQEWTLSRSPVPELKVGIVGNLSSGKSLAVHRYLTGTYVQEESPEGGRFKKEIVVDGQSYLL
LIRDEGGPPELQFAAWVDAVVVFVFSLEDEISFQTVYNYFLRLCSFRNASEVPMLVGTQDAISAANPRVI
DDSRARKLSTDLKRCYYETCATYGLNVERVFQDVAQKVVALRKQQLAIGPCKSLPNSPSHSAVSAASI
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KKAAECKVDSIGSGRAIPIKQGILLKRSGKSLNKEWKKVYTLCDNGLLTYHPSLHDYMQNIHGKEIDL
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GLHQRCSCVSSADQWSEATTSLPPGMQHPASGPAEVLSSSPKLDPPPSPHSNRKHRRKSTGTPRDGP
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LANSVWEAGLGGYSKPGPDACREEKERWIRAKYEQKLFIAPLPSSDVPLGQQLLRAVVEDDLRLLVMLLA
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>gi|5453840|ref|NP_006377.1| DEAD (Asp-Glu-Ala-Asp) box polypeptide 17 isoform 1; probable RNA-dependent helicase p72 [Homo sapiens]
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GKTLAYLLPAIVHINHQPYLERGDGPICLVLAPTRELAQVQQVADDYKGCSRLLKSTCIYGGAPKGQIR
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PKEVRQLAEDFLRDYTQINVGNLELSANHNIQLQIVDVCMESEKDHLIQLMEEIMAENKTIIFVETKR
RCDDLTTRMRRDGWPAMCIHGDKSQPERDWLNEFRSGKAPILIATDASRGLDVEDVKFVINYDYPN
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>gi|4758138|ref|NP_004387.1| DEAD (Asp-Glu-Ala-Asp) box polypeptide 5; DEAD box-5 [Homo sapiens]
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QEVETYRRSKEITVRGHNCPKPVLFNFYEANFPANVMDVIARQNFTPTAIQAGWPVALSGLDMVGVAQT
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IRDLERGVIEICIATPGRLIDFLECKGKTNLRRRTYLVLEADRMLDMGFEPOQIRKIVDQIRPDRQTLMSA
TWPKEVRQLAEDFLDYIHNIGALELSANHNIQLQIVDVCHEKDEKLIRLMEEIMSEKENKTIVFET
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DDRRDRYSAGKRGGFNTFRDRENYDRGYSSLLKRDGFAKTQNGVYSAANYTNGSGSNFVSAGIQTSPRT
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>gi|8922581|ref|NP_060643.1| hypothetical protein FLJ10665 [Homo sapiens]
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PMRLRDPEPEKRHGGHVGAGLLHSPKLKELTKAHELEVRLHTFSMFGMPRLPPEDRRHWEIGEGGDGSLT
IEKSWRELPGHKEMSQECLHQQEALWELLTELIVYVRKLKIMTDLLAAGLLNLQRVGLLMEVAETLFG
NVPSLIRTHRSFWDEVLGPTLEETRASGQPLDPIGLQSGFLTFGQRFHPVYQYCLRVKQTMAYAREQQET
NPLFHAFVQWCEKHRSGRQMLCDLLIKPHQIRITKYPLLLHAVLKRSPEARAQEALNAMIEAVESFLRHI
NGQVRQGEEQESLAAAQRIGPYEVLEPPSDEVEKNLRPSTLDLTSMLGVASEHTRQLLLEGPVVK
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CPSPPTDRAQWLEKTQQAQALQKLKAEEYVQQKRELLTLYRDQDRESPSTRPSTSLEGSQSSAEGRTPE
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QRMRGPHIIQLDTPLSASEV

>gi|29728516|ref|XP_030970.8| similar to hypothetical protein FLJ10665 [Homo sapiens]
MNSVLTKHGSPPRSWLSLCGTDDQSPAEEKGLRCQNPACMDKGRAAKVCHADCQQLHRRGPLNLCEAC
DSKFHSTMHYDGHVRFDLPPQGSVLARNVSTRSCPPRTSPAVDLEEEEEESSVDKGDRKSTGLKLSKKK
ARRRHTDDPSKECFTLKFDLNVDIETEIVPAMKKSLGEVLLPVFERKGIALGKVDIYLDQSNTPSLTF
EAYRGGHYLRVKAFAKPGDEGKVEQGMKDSKSLSLPILRPGTGPALERVDAQSRRESLDILAPGRRR
KNMSEFLGEASIPGQEPPTPSSCLPSGSSGNTGDSWKNRAASRSGFPSSGPSTSAGGREVDKMEQL
EGKLHTYSLFGLPRLPRGLRFHDSEWEYDEDEDEDEDEDEDEDEDEDEDEDEDEDEDEDEDEDEDE
LLHTEASYIRKLRVIINLFLCCLLNQESGLLCEVEAERLFSNIPEIAQLHRRRLWASVMAPVLEKARRTR
ALLQPGDFLKGFKMFGSLFKPYIIRYCMEEEGCMEYMRGLRDNDLFRAYITWAEKHPQCQLRKLSDMLAK
PHQRLTKYPLLKSVLRKTEEPRAKEAVVAMIGSVERFIHHVNACMRQRQERQRLAAVVSRIDAYEVVES
SSDEVDKLKEFLHLDLTAPIPGASPEETRQLLLEGSLRMKEGKDSKMDVYCFLFTDLLLVTKAVKKAER
TRVIRPPLLVDKIVCRELRDPGSFLIYLNEFHSAGAYTFQASQALCRGWDTIYNAQNQLQQLRAQE
PPGSQQPLQSLEEEEEEDEQEEEEEEEEEEDSGTSAASSPTIMRKSQSPDSQHCASDGSTETLAMVV
VEPGDTLSSPEFDSPGPSSQSDETSLTTASSATPTSELLPLGPVDRGSCSMSAAGTLSPTSLQDFVAP
GPMAELVPRAPESPVPSPPPSPRLRRRTPVQLLSCPPHLLSKSEASLLQLLAGAGTHGTPSAPSRSLS
ELCLAVPAPGIRTQGSPQEAGPSWDCRGAPSPGSPGLVGCLAGEPAGSHRKRCGDLPSGASPRVQPEPP
PGVSAQHRKLTQALYRIRTTLLNSTLTASEV

>gi|8923496|ref|NP_060334.1| hypothetical protein FLJ20530 [Homo sapiens]
MAAFLKNVCLGLELDLQYVFMISSEHFLITLLKDEERKLLVDQMRKRSRPNLICKPVTFSYDIPASASVN
IGQLEHQILISVDPWRIRQILIELHGMTSERQFWTVSNKWEVPSVYSGVILGIKDNLTRDLVYILMAKGL
HCSTVKDFSHAKQLFAACLELVTEFSPKLRQVMLNEMLLDIHTHEAGTQAGERPPSDLISRVGYLEM
RLPDIPLRQVIAEECVAFMLNWRNEYLTQVPAFLLQSNPYVKLGQLLAATCKELPGPKESRRTAKDLW
EVVVQICSVSSQHKGNDGRVSLIKQRESTLGIMYRSELLSFIKKREPLVLTIIILSLFAKLHNREDIV
NDITAEHISIWPSSIPNLQSVDFEAVAITVKEVRYTLSINPNNHSLIIQADYIFATNQYSAALHYYLQ
AGAVCSFFNKAQPPDVYTDQVIRKMIKCCSLLNCHTQVAILCQFLREIDYKTAFKSLQEONSHDAMSY
YDYIWDTILEYLTHHKRGETDKRQIAIAKIGOTELNASNPEEVQLAAQRKKFLQAMAKLYF

>gi|4557601|ref|NP_000798.1| gamma-aminobutyric acid A receptor, alpha 2 precursor [Homo sapiens]
MKTKLNIYNIEFLVFVFLWDPARLVLANIQEDEAKNNITIFTRILDRLLDGYDNRLRPGLGDSITEVFT
NIYVTSGPVSDTMEYTDVFRQWKDERLKFKGPMNIRLNNLMASKIWTPTDFFHNGKKSVAHNMT
MPNKLLRIQDDGTLYYTMRLTVQAECPMHELFPMDAHSCPLKFGSYAYTTSEVTYIWTYNASDSVQVAP
DGSRLNQYDLLGQSIGKETIKSSTGEYTVMTAHFLKRKIGYFVICTYLPICMTVILSQVSFWLNRESVP
ARTVFGTTVLTMTTLSISARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGWITWDGKSVV
NDKKEKASVMIQNNAYAVAVAMYAPNLSKDPVLSTISKATTPEPNKKPENKPAEAKTFNSVSKIDRM
SRIVFPVLFGTFLVWATYLNREPVLGVSP

>gi|4503861|ref|NP_000801.1| gamma-aminobutyric acid (GABA) A receptor, alpha 5 precursor [Homo sapiens]

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MDNGMFSGFIMIKNLLLFCISMNLSSHFGFSQMPTSSVKDETNDNITIFTRILDGLLDYDNRLRPGLE
RITQVRTDIYVTSGPVSDEMEYTDIVFFRQSWKDERLRFKGPMQRLPLNNLLASKIWPDTFFHNGKK
SIAHNMTTPNKLLRLEDDGTLLYTMRLTISAECPMQLEDFPMDAHACPLKFGSYAYPNSEVVYWTNGST
KSVVVAEDGSRLNQYHLMGQTVGTENISTSTGEYTIMTAHFHLKRKIGYFVIQTYLPCIMTVILSQVSFW
LNRESVPARTVFGVTTVLTMTTLSIARNSLPKVAYATAMDWFIAVCYAFVFSALIEFATVNYFTKRGWA
WDGKKALEAAKIKKREVILNKSTNAFTTGKMSHPPNIPKEQTAGTSNTTSVSKPSEEKTSESKKTYN
SISKIDKMSRIVFPVLFGTFLVYVATYLNRPEVIKGAAASK

>gi|4504019|ref|NP_000162.1| glycine receptor, alpha 1 [Homo sapiens]
MYSFNTLRLYLSGAIVFFSLAASKEAAARSATKPMPSDFLDKLMGRTSGYDARIRPNFKGPPNVSCN
IFINSFGSIAETTMDYRVNIFLROQWNPDRLAYNEYPDDSLDLDPSMLDIWKPDFFANEKGAFHEIT
TDNKLLRISRNGNVLYSIRITLACPMGLKNFPMDVQTCIMQLESFGYTMMNDLIFEWQEQQAVQADGL
TLPQFILKEEKDLRYCTKHYNTGKFTCIEARFHLEQMGGYLIQMYIPSLLIVLWSWISFWINMDAAPAR
VGLGITTVLTMTTQSSGSRASLPKVSYVKAIIDIWMAVCLLFVFSALLEYAAVNFSRQHKEELLRFRRKRR
HHKEDEAGEGRFNFSAYGMGPACLQAKDGISVKGANNNTNPPPAPSKSPEEMRKLFIQRACKIDKISR
IGFPMAFLIFNMFYWIYKIVRREDVHNQ

>gi|20127457|ref|NP_004121.2| glycogenin [Homo sapiens]
MTDQAFVTLTNDAYAKGALVLGSSLKQHRTTRRLVVLATPQVSDSMRKVLETVFDEVIMVDVLDGDSA
HLLTMKRPPELGVTLTKLHCWSLTQYSKCVFMADTLVLANIDDLFDREELSAAPDPGWPDCFNSGVFVYQ
PSVETYNQLLHLASEQGSFDGGDQGILNTFFFSSWATTDIRKHLFPIYNLSSISIYSYLPFKVFGASAKV
VHFLGRVKPWNVTYDPKTKSVKSEAHDPNMTHPEFLILWVNIFTTNVLPLLQQFGLVKDTCSYVNVLSDL
VYTLAFSCGFCRKEDVSGAISHSLGEIPAMAQPFVSSEERKERWEQQADYMGADSFNDIKRKLDTYLQ

>gi|5453674|ref|NP_003909.1| glycogenin 2 [Homo sapiens]
MSETEFHGAQAGLELLRSSNSNPTSASQSAGMTVDQAFVTLATNDIYCQGALVLGQLRRLTRKLVV
LITPQVSSLLRVILSKVFDDEVIEVNLDIADSADYIHLAFLKRPPELGLTLKLCWTLTHYSKCVFLADATLV
LSNVDELFDRGEFSAAPDPGWPDCFNNSGVFVFPQLSHTHKLLLQHAMEHGSFDGADQGLNSFRNWSTT
DIHKHLFPIYNLSSNTMYTYSPAFKQFGSSAKVVFHLSMKPWNYKYNPQSGSVLEQGSVSSSQHQAAFL
HLNWTVYQNNVLPLYKSVQAGEARASPGHTLCHSDVGGPCADSASGVGEPCENSTPSAGVPCANSPLGSN
QPAQGLPEPTQIVDETLSPERGRSEDMIACPETETPAVITCDPLSQPSQPADFTETETILQPANKVES
VSSEETFEPSPQELPAEALRDPQLQDALEVDLAVSVSQISIEEKVKELSPPEERRKWEGRIDYMGKDAFA
RIQEKLDRFLQ

>gi|30147855|ref|XP_301113.1| similar to Glycogenin-1 [Homo sapiens]
MTDQVFVTLTTNDAYTKGALVLGSSLKQHRTTRRLVVLATPQVSDSMRKVLETVFDEIIVVDVLDGDSA
HLLTMKRPPELAFKVFASAKVVFHLSMKPWNYTYPKTKSVKNESHHPNVTHPEFLILWVNIFTTVLP
LLQQFGLVKDTFSYVNVENVSGAISHSLGGIPAMAQSVYPQKNGRSGGNRARPIIWEQIPLTTSRGSLT
LTSSRNTAFSCGHIHFTSLVSDT

>gi|4757726|ref|NP_004632.1| myeloid/lymphoid or mixed-lineage leukemia
(trithorax (Drosophila; ALL1 fused gene from chromosome 10;
myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila)
homolog); translocated to, 10 [Homo sapiens]
MVSSDRPVSLDEVSHSMKEMIGGCCVCSDERGWAENPLVYCDGHGCSVAHVQACYGIVQVPTGPWFCKR
CESQERAARVRCELCPHKGDKALRKTDNGGWAHVVCALYIPEVQFANVSTMPEPILQSVPHDRYNTKCYIC
DEQGRESKAATGACMTCNKHGCRQAFHVTCAQFAGLLCEEENGADNVQYCGYCKYHFSKLKSKRGSNR
SYDQSLSDSSHSQDKHHEKEKKKYKEDKHKQKHKKQPEPSPALVPSLTVTTEKTYTSTSNNISGSLK
RLEDTTARFTNANFQEVSAHTSSGKDVSERGSEGKGKSSAHSSGQRGRKPGGGRNPGBTVAASPFPO
GSFSGTPGSVKSSSSVQSPQDFLSFTDSLRLNDSYSHSQSSATKDVHKGESGSQEGGVMSFSTLIGL
PSTSATVTSQPKSFENSPGDLGNSSLPTAGYKRAQTSGIIEETVKEKCRKGNKQSXHGPGPRPKGNKNQENV
SHLSVSSASPTSSVASAAGSITSSSLQKSPTLLRNGSLQSLSVGSSPVGSEISMQYRHDGACPTTFSEL
LNAIHNDRGDSSTLTQKELKFIGIYNSNDVAVSFNVVSGSGSSTPVSSSHLPQSSGHLQQVGALPSA
VSSAAPAVATTQANTLSSGSSLQAPSHMYGNRSNSMAALIAQSENNQTDQDLGDSRNLVGRGSSPRGS
LSPRSPVSSLQIYDQPGNSSLLENLPPVAASIEQLLERQWSEGQQFLLLEQGTPSDILGMLKSLHQLQVEN
RRLEEQIKNLTAKKERLQLLNAQLSVPFPITANPSPSHQIHTFSQATPTTDSLNSSKSPHIGNSFLPD
NSLPVLNQDLTSSGQSTSSSALSTPPPAGQSPAQQGSGVSGVQQVNGVTGALASGMQPVTSTIPAVSA

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VGGIIGALPGNQLAINGIVGALNGVMQTPVITMSQNPTPLHTTVPPNATHPMPATLTNSASGLGLLSDQQ
RQILIHQQQFQQLLNSQQLTPVHRRPHFTQLPPTHFSPSMEIMQVRK

>gi|5174577|ref|NP_005928.1| myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to, 6; Myeloid/lymphoid or mixed-lineage leukemia, translocated to, 6; myeloid/lymphoid or mixed-lineage leukemia (trithorax (*Drosophila*) homolog); translocated to, 6 (*Homo sapiens*)

MKEMVGCCVCSDERGWAENPLVYCDGHACSVAVHQCAYGIVQVPTGPWFCRKCESQERAARVRCELCPH
KDGALKRTDNGGWAHVVCALYIPEVQFANVLTMEPIVILQYVPHDRFNKTCYICEETGRESKAASGACMTC
NRHGCRCQAFHVTCAQMAGLLCEEVEVDNVYCGYCKYHFSKMKTSRHSSGGGGGAGGGGGSMGGGGS
GFISGRRSRSASPSTQQEKHPTHHERGQKKSRSRKDERLKQKHKKRPESPSSILTPPVVPTADKVSSASS
SSHHEASTQETSESSRESKGKKSSSHSLSHKGKKLSSKGKVSSFTSASSSSSSSSSSGGPFQPAVSSLQ
SSPDFSAFPKLEQPEEDKYSKPTAPAPSAPPSSPAPEPPKADLFEQKVVSGFGPIMRFSTTSSSGRAR
APSPGDYKSPHVGTGSGASAGTHKRMPALSATPVADETPTETGLKEKKHKASKRSRHPGRPKGSRNKEGT
GGPAAPSLPSAQLAGFTATAASFSGGSLSVSGLGLSSRTFGPSGLPSLSLESPLLGAGIYTSNKDPI
SHSGGMLRAVCSTPLSSSLGGPGTSALPRLSRSPFTSTLPSSASISTTQVFSLAGSTFSLPSTHIFGT
PMGAVNPILLSQAESSHTEPDLEDSCFRCRGTSQESLSSMSPISSLPAFDQTAAPCGGGQLDPAAPGT
TNMEQLLEKQGDGEAGVNIVEMLKALHALQKENQRLQEQQIQLSLTAKKERLQIINVQLSVPFPALPAALPA
ANGPVPGPYGLPPQAGSSDSLSTSNSPGKSSGLDNSLSTSSEDPHSGCPRSSSSLSFHSTPPPLPLL
QQSPATLPLALPGAPAPLPPQPNQGLGRAPGAAGLGLGAMPMAEGLLGGLAGSGGLPLNGLLGGLNAAAPN
PASLSQAGGAPTLQLPGCLNSLTEQQRHLLQQQEQQLQQLQQLASPQLTPEHQTVVYQMIQQIQQKREL
QRLOMAGGSQLPMASLLAGSSTPLLSAGTPGLLPTASAPPPLPAGALVAPSLGNNTSLMAAAAAAAVAA
AGGPPVLTATNPFLSLSGAEGGGGPKGGTADKGASANQEKG

>gi|4505957|ref|NP_002688.1| POU domain, class 2, transcription factor 1; Octamer-binding transcription factor-1 [*Homo sapiens*]
MNNPSETSKPSMESGDGNTGTQTNGLDFQKQPVPGGAISTAQAQAFGLHLHQVQLAGTSLQAAAQSLNV
QSKSNEESGDSQQPSQPSQPSVQAAIPQTQLMLAGGQITGLTLPAQQQQLLQQAAQAAQQLAAAVQOH
SASQQHSAAGATISASAATPMTOPIQLSQQPIQIAQDLQQLQQLQQQNLNLQQFVLVHPTTNLQPAQFIISO
TPQGQQQLLQAQNLTQLPQQSQANLLQSQPSITLTSQPATPTRTIAATPIQTLPQSQSTPKRIDTPSLE
EPSDLEELQFAKTFQRRRIKLGFTQGDVGLAMGKLYGNDFSQTTISRFEALNLSFKNMCKLKPPLKEWL
NDAENLSSDSSLSSPSALNSPGIEGLSRRRKRTSIETNIRVALEKSFLENQKPTSEEITMIADQLNMEK
EVIRWFNCNRRQKEKRINPPSSGTSSSPIKAIFPSPTSLVATTPSLVTSSAATLTVSPVPLTSAAVT
NLSVTGTSDDTSNNATVISTAPPASSAVTSPSLSPSPSASASTSEASSASETSTTQTTSTPLSSPLGTS
QVMVTASGLQAAAAALQGAAQLPANASLAAMAAAAGLNPNSMAPSQAAGGALLSLNPGTLSGALSPAL
MSNSTLATIQLASGGSLPITSLDATGNLVFANAGGAPNIVTAPLFLNPQNLSLTSNPVSLVSAAAASA
GNSAPVASLHATSTSAESIQNSLFTVASASGAASTTTASKAQ

>gi|4505959|ref|NP_002689.1| POU domain, class 2, transcription factor 2 [*Homo sapiens*]
MVHSSMGAPEIRMSKPLEAEKQGLDSPSEHTDTERNGPDTNHQNPQNKTSPFSVSPGPSTKIKAEPSG
DSAPAAPLPPQPAQPHLPQAQMLMTGSQLAGDIQQLLQQQLVLPVGHHLQPPAQLPQAQQSQPGLLP
TPNLFQLPQQTQGALLTSQPRAGLPTQPPKCLEPPSHPEEPSDLEELQFARTFKQRRRIKLGFTQGDVGL
AMGKLYGNDFSQTTISRFEALNLSFKNMCKLKPPLKEWLNDAEITMSVDSSLPSPNQLSSPSLGFGLPGR
RRKKRTSIETNVRFALEKSFLANQKPTSEEILLIAEQLHMEKEVIRWFNCNRRQKEKRINPCSAAPMLPS
PGKPASYSPHMVTQGGAGTLPQLSQASSSLTTVTTLSAVGTLHPSRTAGGGGGGGAAPPLNSIPSVT
PPPPATTNSTNPSHQGSHSAIGLGLNPSTGPGLWWNPAPYQP

>gi|7657409|ref|NP_055167.1| POU transcription factor; likely ortholog of mouse POU domain, class 2, transcription factor 3 [*Homo sapiens*]
MVNLESMHTDIKMSGDVADSTDARSTLSQVEPGNDRKGLDFNRQIKTEDLSDSLQQTLSHRPCHLSQGPA
MMSGNQMSGLNASCQDMASLHPLQQLVLVPGHLQSVSQFLLSQTQPGQQGLQPNNLLPFPQQQSGLLLPO
TGPGLASQAFGHPGLPGSSLEPHLEASQHLPVPKHLPSSGGADEPSDLEELKFAKTFKQRRRIKLGFTQG
DVGLAMGKLYGNDFSQTTISRFEALNLSFKNMCKLKPPLKEWLNDAESSPSDPSVSTPSSYPSLSEVFGR
KRKKRTSIETNIRLTLEKRFQDNPKPSSEEISMIAEQLSMEKEVVRVWFNCNRRQKEKRINCPVATPIKPP

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VYNSRLVSPSGSLGPLSVPPVHSTMPGTVTSSCPGNNSRPSSPGSGLHASSPTASQNNSKAAVNSASSF
NSSGSWYRNHSTYLH

>gi|4506071|ref|NP_002731.1| protein kinase C, iota [Homo sapiens]
MSHTVAGGGSGDHSHQVRVKAYYRGDIMITHEPEPSISFEGLCNEVRDMCSFDNEQLFTMKWIDEEGDPCT
VSSQLEEEAFRLYELNPKDSELLIHVFPVPERPGMPCPGEDKSIYRRGARRWRKLYCANGHTQAKRFN
RRAHCAICTDRIWGLGRQGYCINCKLLVHKCHKLVTIECGRHSLPQEPPVMPMDQSSMHSDHAQTVIPY
NPSSHESLDQVGEKEAMNTRESGKASSSLGLQDFDLLRIGRGSYAKVLLVRLKKTDRYAMKVVKEL
VNDDEDIDWVQTEKHFVFEQASNHPFLVGLHSCFQTESRFFFVIEYVNGGDLMFHMQRQRKLPEEHARFYS
AEISLALNYLHERGIYRDLKLDNVLLDSEGHIKLTDYGMCKEGLRPGDTTSTFCGTPNYIAPEILRGED
YGFSDVWWALGVLFEMMAGRSPFDIVGSSDNPQNTEDYLQVILEKQIRIPRSLSVKAASVLSFLNK
DPKERLGCHPQTGFADIQGHPPFRNVDWDMMEQKQVVPPFKPNISGEFGLDNFDSQFTNEPVQLTPDDDD
IVRKIDQSEFEGFEYINPLLMSAECV

>gi|10864650|ref|NP_002735.2| protein kinase C, zeta [Homo sapiens]
MPSRTDPKMEGSGGRVRLKAHYGGDIFITSVDAATTFEELCEEVRDMCRLHQHQPLTLKWDSEGDPCTV
SSQMELEEEAFRLARQCRDEGLIIVFPSTPEQPGPLCPGKEDKSIYRRGARRWRKLYRANGHLFQAKRFRN
RAYCGQCSEWIWGLARQGYRCINCKLLVHKRCHGLVPLTCRKHMDSVMPSEQPPVDDKNEDADLPSEETD
GIAYISSSRKHDSDIKKDDSEDLKPVIDGMDGIKISQGLGLQDFDLIRVIGRGSYAKVLLVRLKKNDQIYAM
KVVKKELVHDDDEDIDWVQTEKHFVFEQASNPNFLVGLHSCFQTTSRFLVIEYVNGGDLMFHMQRQRKLPE
EHARFYAAEICIALNFLHERGIYRDLKLDNVLLDADGHIKLTDYGMCKEGLGPGDTTSTFCGTPNYIAP
EILRGEEYGFSDVWWALGVLFEMMAGRSPFDIITDPDMNTEDYLQVILEKPIRIPRFLSVKASHVLK
GFLNKDPKERLGCRPQTGFSDIKSHAFFRSIDWDLLEKKQALPPFQPQITDDYGLDNFDTQFTSEPVQLT
PDDEDAIKRIDQSEFEGFEYINPLLSTEESV

>gi|4759124|ref|NP_004162.1| solute carrier family 1, member 2;
H.sapiens mRNA for glutamate transporter; glutamate/aspartate
transporter II; excitatory amino acid transporter 2; glial high
affinity glutamate transporter [Homo sapiens]
MASTEGANNMPKQVEVRMPDSHLGSEEPKHHRLGLRICKNLNLLLTIVFGVILGAVCGGLLRLASPI
HPDVVMLIAFPGDILMRMLKMLIPLISSLITGLSGLDAKASGRLGTRAMVYMSSTIIIAAVLGVILVL
AIHPGNPKLKKQLGPQKKNDEVSSLDALRNLFPENLVQACFQQIQTVTKVLVAPPDDEEANATSA
EVSSLNETVTEVPEETKVMVIKKGLEFKDGMNVLGLIGFFIAFGIAMGKMGDQAKLMDFFNILNEIVMKL
VIMIMWYSPPLGIACLICGKIIIAIKDLEVARQLGMYMVTVIIGLIIHGGIFLPLIYFVVTRKNPFSLFAG
IFQAWITALGTASSAGTLPVTFRCLEENLGIDKRVTRFVLPVGATINMDGTALYEAVAAIFIAQMNGVVL
DGGQIVTVSLTATLASVGAASIPSAGLVTMILLITAVGLPTEDISLLVAVDWLLRMRTSVNVVGDSFGA
GIVYHLSKSELDTIDSQHRVHEDIEMTKTQSIYDDMKNHRESNSNQCVYAAHNSIVDECKVTLAANGKS
ADCSVEEWPWREK

>gi|4759126|ref|NP_004163.1| solute carrier family 1 (glial high
affinity glutamate transporter), member 3 [Homo sapiens]
MTKSNGEEPKMGRMERFQQGVRKRTLLAKKKVQNITKEDVKSYLFRNAFVLLVTAVIVGTLGFTLRP
YRMSYREVKYFSFPGEMLRMLQMLVPLISSLVTGMAALDSKASGKMGMRVYVYMTTTIIAVVIGII
IVIIHPGKGTKENMIREGKIVRVTAAADFLLIRNMFPNLVEACFKQFKTNYEKRSFKVPIQANETLV
GAVINNVSEAMETLTRITEELVPVPGSVNGVNALGLVFSMCFGFVIGNMKEQGQALREFFDSLNEAIMR
LVAVIMWYAPVGILFLIAGKIVEMEDMGVIGGQLAMYTVTIVGLLIHAVIVPLLYFLVTRKNPFWFIG
GLLQALITALGTSSSATLPIFKCLEENNGVDKRVTRFVLPVGATINMDGTALYEALAAIFIAQVNNFE
LNFGQIITISITATAASIGAAGIPQAGLVTMIVLTSVGLPTDDITLIIAVDWFLDRLRTTNVLGDSLG
AGIVEHLSRHELKNRDVEMGNVIEENEMKKPYQLIAQDNETEKPIDSETKM

>gi|20070239|ref|NP_006662.2| solute carrier family 1 (glutamate
transporter), member 7; excitatory amino acid transporter 5 (retinal
glutamate transporter) [Homo sapiens]
MVPHAILARGRDVCRNRGLLILSLSVIVGCLLGFRLRRLSPQEISYFQFPGEMLMRMLKMMILPLVV
SSLMSGLASLDAKTSSRLGVLTAYYLWTTFMAVIVGIFMVSIIHPGSAAQKETTEQSGKPIMSSADALL
DLIRNMFPANLVEATFKQYRTKTPVVKSPKVAPPEAPRRIIYGVQEENGSHVQNFALDLTPPPEVYY
KSEPGTSDGMNVLGIVFFSATMGIMLGRMGDSGAPLVSFCQCLNESVMKIVAVAVWYFPFGIVFLAGKI

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LEMDDPRAVGKKLGFYSVTVVCGLVLHGLFILPLLYFFITKKNPIVFIRGILQALLIALATSSSATLPI
TFKCLLENHHIDRRRIARFVLPVGATINMDGTALYEAVAAIFIAQVNYYELDFGQIITISITATAASIGAA
GIPQAGLVTMVIVLTSVGLPTDDITLIAVDWALDRFRTRMINVLGDAALAAGIMAHICRKDFARDTGTEKL
LPCETKPVSLQEIWAQONGCVKSVAEASELTGLGPTCPHVPVQVEREELPAASLNHCTIQISELETNV

>gi|21314632|ref|NP_003029.2| solute carrier family 1, member 4;
glutamate/neutral amino acid transporter;
alanine/serine/cysteine/threonine transporter [Homo sapiens]
MEKSNETNGYLDQAQAGPAAGPGAPGTAAGRARRCAGFLRRQALVLLTVSGVLAGAGLGAALRGLSLSRT
QVTYLAFFGEMLLRMLRMIIILPLVVCSLVSAGAASLDASCLGRLGGIAVAYFGLTTLSSASALAVALAFIIK
PGSGAQTQLQSSDLGLEDGPPPVPKETVDSFLDLARNLFPNSNLVVAAFRTYATDYKVVTQNSSSGNVTHE
KIPIGTEIGMNLIGLVLFALVLGVALKKLGSEGEDLIRFFNSLNEATMVLVSWIMWYVPVGIMFLVGSK
IVEMKDIIVLVTSLGKYIFASILGHVIHGGIVLPLIYFVFTRKNPFRFLGLLAPFATAFATCSSSATLP
SMMKCIEENNGVDKRISRFILPIGATVNMDGAAIFQCVAAVFIQLNNVELNAGQIFTILVTATASSVGA
AGVPAGGVLTIAIILEAIGLPTHDLPLILAVDWIVDRTTVNVEGDAKGAGILHHLNQKATKKGEQELA
EVKVEAIPNCKSEEETSPLVTHQNPAGPVASAPELESKESVL

>gi|4827012|ref|NP_005062.1| solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6; excitatory amino acid transporter 4 [Homo sapiens]
MSSHGNSLFLRESGQRLGRVGWLQLQESLQQRALRTRLRLQTMTEHVLRLRRNAFILLTVSAVIVG
SLAFALRPYQLTYRQIKYFSFPGEMLMRMLQMLVLPLIVSSLVTGMASLDNKATGRMGMRAAVYYMVTI
IAVFIGILMVTIIHPGKGSKEGLHREGRIETIPTADAFMDLIRNMFPNLVEACFKQFKTQYSTRVVT
MVRTEENGSEPGASMPPFSENGTSFLENVTRALGTQEMLSFEETVPVPGSANGINALGLVVFSAVGL
VIGGMKHKGVRVLRDFDSLNEAIMRLVGIIIWYAPVGILFLIAGKILEMEDMAVLGGQLGMYTLTVIVGL
FLHAGIVLPLIYFLVTHRNPFPFIGGMLQALITAMGTSSSATLPTIFRCRCLEEGLGVDRRIITRFVLPVGA
TVNMDGTALYEALAAIFIAQVNYYELNLQGITISITATAASVGAAGIPQAGLVTMVIVLTSVGLPTEDI
TLIIAVDWFLDRLRTMTNVLGDSIGAAVIEHLSQRELELQEAELTSLGKPYKSLMAQEKGASRGRGGN
ESAM

>gi|18252049|ref|NP_004161.2| solute carrier family 1, member 1; excitatory amino acid transporter-3; excitatory amino acid carrier 1 [Homo sapiens]
MGKPARKGCEWKRFKNNWVLLSTVAAVVLGITTGVLVREHSNLSTLEKFYFAFPGEILMRLKLIIPL
IISSMITGVAALDSNVSGKIGLRAVYYFCTTLIAVILGIVLVSIKPGVTQKVGEIARTGSTPEVSTVD
AMLDLIRNMFPENLVQACFQQYKTKREEVKPPSDPEMMTEESFTAVMTTAISKNKTKEYKIVGMYSDGI
NVLGLIVFCLVFGLVIGKMGEGKQILVDFPNALSDATMKIVQIIMCYMLGLILFLIAGKIIEVEDWEIFR
KLGLYMATVLTGLAHSIVLPLIYFIVVRKNPFRFAMGMAQALLTALMISSSSATLPVTFRCAEENNQV
DKRITRFVLPVGATINMDGTALYEAVAAVFIQLNDLGLGIGQIITISITATSASIGAAGVPQAGLVTMV
IVLSAVGLPAEDVTLIIAVDWLLDRFRTMVNVLGDAFGTGIVEKLSKKEEQMDVSSEVNIVNPFAEST
ILDNEDSDTCKSYVNGGFAVDKSDTISFTQTSQF

>gi|5032093|ref|NP_005619.1| solute carrier family 1 (neutral amino acid transporter), member 5; neutral amino acid transporter B; RD114 virus receptor; baboon M7 virus receptor [Homo sapiens]
MVADPPRDSKGLAAAEPТАNGLALASIEDQAAAGGYCGSRDQVRCLRANLLVLLTVVAVVAGVALGL
GVSGAGGALALGPERLSAFVFPGEPLLRLRMIILPLVVCSLIGGAASLDPGALGRLGAWALLFLVTTL
LASALGVGLALALQPGAASAAINASVGAAGSAENAPSKEVLDSDLARNIFPSNLVSAFRSYSTTYEE
RNITGTRVKPVGQEVGMNILGLVVFAIVFGVALRKLGPEGELLIRFFNSFNEATMVLVSWIMWYAPVG
IMFLVAGKIVEMEDVGLLFARLGKYLCCLLGHAIHGLLVLPLIYFLFTRKNPFRFLWGIVTPLATAFGTT
SSSSATLPLMMKCVEENNGVAKHISRFILPIGATVNMGAALFQCVAAVFIAQLSQQSLDFVKIITILVT
ATASSVGAAGI PAGGLTLAIILEAVNLPVDHISLILAVDWLVDRSCTVLNVEGDA
LGA
GAGL
LQNY
VDRTE
SRSTEPELIQVKSELPDLPVPTEEGNPLLKHYRG
PAGDATVASEKE
SVM

>gi|4507047|ref|NP_003036.1| solute carrier family 7 (cationic amino acid transporter, y⁺ system), member 1; amino acid transporter, cationic 1; ecotropic retroviral receptor [Homo sapiens]

MGCKVLLNIGQQMLRRKVVDCSREETRLSRCNTFDLVALGVGSTLGAGVYVLAGAVARENAGPAIVISF
 LIAALASVLAGLCYGEFGARVPKTGSAYLYSYVTVGELWAFITGWNLILSYIIGTSSVARAWSATFDELI
 GRPIGEFSRTHMTLNAPGVLAEADPDIYAVIILILITGLLTGKVESAMVNKIFTCINVVLVLFIMSGFV
 KGSVKNWQLTEEDFGNTSGRLCLNNDTKEGKPGVGGFMPFGSGVLSGAATCFYAFVGFDCAATTGEEVK
 NPQKAIPVGIVASLLICFIAYFGVSAALTLMMPYFCLDNNSPLPDAFKHVGWEGAKYAVAVGSLCALSAS
 LLGSMFPMPPRVIYAMAEDGLLFKFLANVNDRTKTPIIATLASGAVAAMAFLFDLKDLDLMSIGTLAY
 SLVAACVVLRLYQPEQPNLVYQMASTSDELDPADQNELASTNDSQLGFLPEAEMFSLKTILSPKNMEPSK
 ISGLIVNISTSLIAVLIITFCIVTGLREALTKGALWAVFLLAGSALLCAVVTGVIWRQPESKTKLSFKV
 PFLPVLPILSIFVNVYLMQLDQGTWVRFAVWMLIGFIYFGYGLWHSEEASLDADQARTPDGNLDQCK

>gi|4507049|ref|NP_003037.1| solute carrier family 7 (cationic amino acid transporter, γ system), member 2; amino acid transporter, cationic 2 [Homo sapiens]
 MIPCRAALTFARCLIRRKIVTLDLEDTKLCRCLSTMIDLIALGVGSTLGAGVYVLAGEVAKADSGPSIVV
 SFLIAALASVAGLCYAEFGARVPKTGSAYLYTYVTVGELWAFITGWNLILSYVIGTSSVARAWSGTDFE
 LLSKQIGQFLRTYFRMNYTGLAEYPDFFAVCLLLLGLLSFGVKESAWVNKVFATVNILVLLFVMVAGF
 VKGNVANWKISEEFLKNISASAREPPSENGTSIYGAGGFMPYGFTGTLAGAATCFYAFVGFDCAATTGEE
 VRNPQKAIPIGIVTSSLVCFMAYFGVSAALTLMMPYLLDEKSPLPVAFEYVGWGPACYVVAAGSLCALS
 TSLLGSMFPLPRILFAMARDGLLFRFLARVKRQSPVAATLTAGVISALMAFLFDLKALVDMMSIGTLMA
 YSLVAACVVLRLYQPGLSYDQPKCSPKDGLGSSPRVTSKSESQVTMLQRQGFSMRTLFCPSLLPTQQSA
 SLVSFLVGFLAFLVGLSVLTTYGVHAITRLEAWSLALLTLFLVLFVAIVLTIWRQPQNQQKVAFMVPFL
 PFLPAFSILVNIYLMVQLSADTWVRFSIWMAIGFLYFSYGIHSLEGHLRDENNEEDAYPDNVHAAAEE
 KSAIQANDHHPRNLSSPFIFHEKTSEF

>gi|17939406|ref|NP_116192.2| solute carrier family 7 (cationic amino acid transporter, γ system), member 3 [Homo sapiens]
 MPWQAFRRFGQKLVRRTLESMAETRLARCLSTLDLVALGVGSTLGAGVYVLAGEVAKDKAGPSIVICF
 LVAALSSVLAGLCYAEFGARVPRSGSAYLYSYVTVGELWAFITGWNLILSYVIGTASVARAWSAFDNLI
 GNHISKTLQGSIALHVPVHLAEYPDFFALGLVLLLTGLLALGASESALVTKVFTGVNLLVLFVMSIGFV
 KGDVHNWLKTEEDYELAMEALNDTYSLGPLGSGGFVPGFEGILRGAATCFYAFVGFDCAATTGEEAQNP
 QRSIPMGIVISLSCVFLAYFAVSSALTLMMPYVQLOPESPLPEAFLYIGWAPARYVVAVGSLCALSTSLL
 GSMFPMPPRVIYAMAEDGLLFRVLAIRHTGTRTPIIATVSGIIIAAFMAFLFKLTDLVDLMSIGTLAYSL
 VSICVVLRLYQPDQETKTGEEVELQEEAITTESEKLTWLWGLFFPLNSIPTPLSGQIVYVCSSLLAVLLTA
 LCLVLAQWSVPLSGDLLWTAVVVLRLIIGIIVVIVRQPQSSTPLHFKVPAPLLPLMSIFVNLYLMM
 QMTAGTWARFGVWMLIGFAIYFGYGIQHSLEEIKSNQPSRKSRAKTVLDLPGTLVHSV

>gi|29789100|ref|NP_061325.1| similar to glucosamine-6-sulfatases; sulfatase 2 [Homo sapiens]
 MGPPSLVLCLLSATVFSLLGGSSAFLSHHRLKGRFQRDRRNIRPNIIVLTDQDVELGSMQVMNKTRRI
 MEQGGAHFINAFVTPMCCPSRSSILTGKVHNHNTYTNNECSSPSWQAHESRTFAVYNSTGYRTAF
 FGKYLNEYNGSYVPPGKEWVGLLKNSRFYNTLRCNGVKEKHGSDYSKDYLTDLITNDVSFFRTSKKM
 YPHRPVLMVISHAAPHGPEDSAQYQSRLFPNASQHITPSYNYAPNPDKHWIMRYTGPMPKPIHMEFTNMLQ
 RKRLQTLMSVDDSMETIYNMLVETGELDNTYIVYTADHGYHIGQFGLVKGKSMPPYEFDIRVPFYVRGPNV
 EAGCLNPVHIVLNIDLAPTILDIAGLDIPADMDGKSILKLLDTERPVNRFHLKKKMRVWRDSFLVERGKLL
 HKRDNDKVDQAQEEFLPKYQRVKDLCQRAEYQTACEQLGQKWCQVEDATGKLKLHKCKGPMRLGGSRAL
 NLVPKYYGQGSEACTCDSGDYKLSLAGRRKLFKKYKASYVRSRSIRSVAIEVDGRVYHVGLGAAQPR
 NLTKRHWPAGEDQDDKDGDFSGTGGLPDYSAAPIKVTHRCYILENQVQCDLDLYKSLQAWKDHKLH
 IDHEIETLQNQKIKNLREVRGHLKKRPEECDCHKISYHTQHKGRKLHKGSSLHPRKGLQEKDVKWLLRE
 QKRKKKLRKLLKRLQNNDTCSPGLTCFTHDNQHWQTAPFWTLGPFCACTSANNTNTYWCMTTINETHNFL
 FCEFATGFLEYFDLNTDPYQLMNAVNTLDRDVLNQLHVQLMELRSCKGKQCNPRTRNMDLKDGGSYE
 QYRQFQRRKWPPEMKRPSKSLGQLWEGWEG

>gi|29789064|ref|NP_055985.1| sulfatase FP [Homo sapiens]
 MKYSCCALVLAVLGTELLGSLCSTVRSPRFRGRIQQERKNIRPNIIILVPTDDQDVELGSLQVMNKTRKIM
 EHGGATF1NAFVTTPMCCPSRSSMLTGKVHNHNVYTNNECSSPSWQAMHEPRTFAVYNNTGYRTAFF
 GKYLNEYNGSYIIPPGREWGLIKNNSRFYNTVCRNGIKEKHFDFYAKDYFTDLITNESINYFKMSKRYM
 PHRPVMMVISHAAPHGPEDSAQFSKLYPNASQHITPSYNYAPNMDKHWIMQYTGPMPLPIHMEFTNILQR

EX03-053P

KRLQTLMSVDDSVERLYNMLVETGELENTYIYTADHGYHIGQFGLVKGKSMYDFDIRVPFFIRGPSVE
PGSIVPQIVLNIDLAPTIIDIAGLDTPPDVGKSVLKLLDPEKPGNRFRNTKKAKIWRDTFLVERGKFLR
KKEESSKNIQQSNHLPKYERVKELCQQARYQTACEQPGQKWCIEDTSGKLRHKCKGPDLLTVRQSTR
NLYARGFHDKDKECSCRESGYRASRSQRKSQRQFLRNQGTPKYKPRFVHTRQTRSLSVFEGEIYDINLE
EEEELQVLQPRNIAKRHDEGHKGPRDLQASSGGNRGRMLADSSNAVGPPTTVRVTWKCFILPNDSIHCR
ELYQSARAWKDHKAYIDKEIEALQDKIKNLREVRGHLRKPEECSCSKQSYYNKEGVKKQEKLKSKLH
PFKEAAQEVDSKLQLFKENNRRRKERKEKRQRKGEECSPGLTCFTHDNNWQTAPEFWNLGSFCACTS
SNNNTYWCLRTVNETHNLFCEPATGFLEYFDMNTDPYQLNTVHTVERGILNQLHVQLMELRSCQGYKQ
CNPRPKNLDVGNKDGGSYDLHRGQLWDGWEG

>gi|27597096|ref|NP_775491.1| uridine phosphorylase-2; liver-specific
uridine phosphorylase [Homo sapiens]
MASVIPASNRSMRSRNTYVGKRFVHVKNPYLDLMDEDIYLHDLGKTKTHNLPAMFGDVKFVCVGGSPNR
MKAFAFLFMHKELGFEAEEDIKDICAGTDRCMYKTGPVLAISHGMGIPSISIMLHELIKLLHHARCCDV
TIIIRIGTSGGIGIAPGTVVITDIAVDSFFKPRFEQVILDNIVTRSTELDKELEELFNCSEIPNFP TLV
GHTMCTYDFYEGQGRLDGALCSFREKKLDYLKRAFKAGRNIEMESTVFAAMCGLCGLKAAVVCVTL
LDQCDQINLPHDVLYEYQQRPQLLISNFIRRLGLCD

>gi|4507839|ref|NP_003355.1| uridine phosphorylase [Homo sapiens]
MAATGANAekaeshndCPVRLNPNIAKMKEDILYHFNLTSRHFNPALFGDVKFVCVGGSPSRMKAFIR
CVGAELGLDCPGRDYPNICAAGTDRYAMYKVGVLVSHGMGIPSISIMLHELIKLLYYARCSNTIIRIG
TSGGIGLEPGTVVITEQAVDTCFKAEEFEQIVLGKRVIRKTDLNKKLVQELLCASELSEFTTVVGNTMCT
LDFYEGQGRLDGALCSYTEKDKQAYLEAAAGVRNIEMESSVFAAMCSACGLQAAVVCVTLNRLEGDQ
ISSPRNVLSEYQQRPQLLVSYFIKKKLSKA

>gi|5803133|ref|NP_006825.1| RAB32, member RAS oncogene family [Homo
sapiens]
MAGGGAGDPGLAAAAAPAPETREHLFKVLVIGELGVGKTSIIKRYVHQLFSQHYRATIGVDFALKV
LNWD SRTLVRQLWLDIAGQERFGNMTRVYYKEAVGAFVVFDISRSSTFEAVLKWKSDLD
SKVHLPNGSPIPAVL LANKCDQNKDSSQSPSVDQFCKEHGFAGWFETSAKDN
NIEEAARFLVEKILVNHQSFPNEENDV
DKIK LDQETLRAENKSQCC

>gi|11641237|ref|NP_071732.1| RAB38; Rab-related GTP-binding protein
[Homo sapiens]
MQAPHKEHLYKLLVIGDGVGKTSIIKRYVHQNFSHYRATIGVDFALKVLHWD
PETVVRQLWLDIAGQE RFGNMTRVYYREAMGAFIVFDVTRPATFEAVAK
WKNDLDSKLSLPGKPV
VLLANKCDQGKDVL
MNNG LKMDQFC
KEHGFAGWFETSA
KENINIDEASRCLV
KHILANECDL
MESIEPDV
VKPHLT
STKV
ASC
SGCA
S

>gi|4506375|ref|NP_003920.1| RAB7, member RAS oncogene family-like 1
[Homo sapiens]
MGSRDHLFKVLVVGDAAVGKTSLVQRYSQD
SF SKHYKSTVGVDFALKV
LQWS
DYEIVR
LQLW
DIAGQ
ERF TSM
TRLY
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TNAT
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>gi|8923042|ref|NP_060103.1| chromosome 6 open reading frame 37;
retinal expressed gene C6orf37 [Homo sapiens]
MAEGEGYFAMSEDELACSPYIPLGGDFGGGDFGGGDFGGGGFGGHCLDYCESPTAHCN
LNWEQVQR
LDGILSETIPIHGRGN
FPTLELQPSLIVKV
VRRRLAEK
RIGV
RDV
RLNG
SAASH
VLHQ
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MAEESSCTRDCMSFSVLNWDQVSRLHEVLTEVVPIHGRGNFPTLEITLKDIVQTVRSLLEEAGIKVHDVR
LNGSAAGHVLVKDNGLGCKDLDLIFHVALPTAEFQLVRDVVLCSLLNFLPEGVNKLKISPVTLKEAYVQ
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IGESMYGDFEEAFDHLQNRLIATKNPEEIRGGGLLKYSNLLVRDFRPTDQEIKTLERYMCSSRFFIDFPD
ILEQQRKLETYLNQNHFAEEERSKYDYLMLRNVNESTVCLMGHERRQTLNLISLLALRVLAEQNIIPSA
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FEFSIDSFQIILDSLLLFGQCSSTPMSEAFHPTVTGESLYGDFTEALEHLRHRVIATRSPEEIRGGGLK
YCHLLVRGFRPRPSTDVRALQRYMCSRFFIDFPDLVEQRRTLERYLEAHFGGADAARRYACLVTLHRVVN
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LPCN

>gi|22749287|ref|NP_689843.1| hypothetical protein MGC26999 [Homo
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ESYLHNHFIGEGMTKYDYLMTLHGVVNESTVCLMSYERRQILHLITMMALKVLGELNILPNTQKVTCFYQ
PAPYFAAEARYPIYVIPEPPPVSFQPYHPLHFRGSNGMS

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